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A Quantitative Study of Shoot Development in *Vicia faba*

III. The Dry Weights of the Plumular Internodes

BY

K. J. DORMER

(*University of Manchester*)

With five Figures in the Text

ABSTRACT

The development of the bean shoot has been studied by cutting a piece out of each internode and determining the dry weight per unit stem length, the 'linear density' of the internode.

The measurements of this quantity are used in the mathematical analysis of physiological age. It is shown that there is a drastic reduction in relative growth rate of the stem during the unfolding of the ninth leaf and that there is evidence of an unknown factor affecting many of the shoot-growth processes with a maximum intensity in the fifth internode. The bearing of these facts on the conception of the plastochrone as a unit of shoot growth is discussed.

IT was shown in the first paper of this series (Dormer, 1950) that there are characteristic differences in the course of xylem development between successive internodes of the bean plumule. For this purpose the growth of the xylem was followed by counting the cells in transverse sections, a method which has many disadvantages, and which becomes intolerably laborious as soon as interfascicular xylem begins to appear.

As the most obvious change taking place in the developing xylem is the deposition of large quantities of cell-wall substance, it is natural to suppose that it might be possible to follow the progress of events within the stem by observing the resulting changes in dry weight. The dry weight of a given stem segment depends on its length, as well as on the amount of substance present in a transverse section, so that it is necessary to work in terms of dry weight per unit length. This quantity, determined for a piece of stem cut out of the middle of an internode, will be called the *linear density* of that internode. It will be denoted by W_n, where n is the serial number of the internode, and it will be expressed throughout in g. cm.$^{-1}$

The determinations are carried out as follows. Taking a bean plant grown in the same way as those used in previous investigations of this series, its plastochrone age is recorded according to the convention already introduced (Dormer, 1950, p. 424). Using a piece of square brass tubing sharpened at one end in the manner of a cork-borer, a test-piece is then stamped out of the middle of each internode, starting with the third and continuing towards

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the apex as far as possible. The length of each test-piece is equal to the internal diameter of the cutter (2·30 cm.). The pieces are individually numbered, dried for a few days in an oven at 65–70° C., and weighed on a torsion balance sensitive to 2×10^{-4} g.; division by 2·3 then converts the balance readings into linear densities.

The present paper is thus based on a table showing the linear densities of the various internodes of many plants of known plastochrone ages.

It so happens that the linear density of an internode behaves, mathematically speaking, in a more regular manner than any of the other variables which have so far been investigated. As a result, a new and powerful method of analysis becomes available, a method which has been known for some time as an interesting theoretical possibility, but which could not previously be put into practice because no quantity was known which satisfied the required conditions. Before the significance of the procedure can be properly appreciated it will be necessary to examine rather closely some of the mathematical principles which govern the quantitative study of developmental shoot morphology.

The quantitative investigation of the development of a shoot can be carried out in two different ways. In the first place, a particular dimension, such as the length of a selected internode, can be measured at intervals and the measurements plotted against time. This operation constitutes a purely physiological research, the results being characteristic of the conditions under which they are obtained rather than of the nature of the material observed. Alternatively, measurements of one dimension may be plotted, not against time, but against simultaneous observations of some other dimension. Graphs of this kind must be regarded as part of the subject-matter of developmental morphology, since their form will be primarily an expression of the pattern or distribution of growth. Obviously, for example, a graph showing the relationship between the length and breadth of a growing leaf is merely an ontogenetic generalization of the static observation of the length–breadth ratio of the mature leaf. The properties of physiological growth-curves are fairly fully understood. It can, for instance, be predicted that a graph showing how the area of a growing leaf is related to the passage of time will be of a sigmoid form, and that a rise in temperature, within certain limits, will result in an increase in the gradient of the curve. But if the area of the leaf were plotted against the length of its petiole a morphological curve would be obtained, and our ignorance of such phenomena is too great to allow of any prediction either of the general form of the curve or of the effects of temperature changes.

The task of the physiologist is greatly simplified by the availability of a universal external scale of time, which can be applied as a scale of abscissae to any graph representing processes of growth or development, and which is capable, at least in theory, of indefinite extension and subdivision. In morphological work, on the other hand, a suitable scale of abscissae has to be selected from among all the innumerable measurable properties of the plant. The particular quantity which is chosen is said to be employed as a measure of physiological age; it is a measure, that is to say, of the stage of development which a plant has reached, irrespective of the time which may have elapsed since growth began. It is evident that the form of the graphs obtained from observations on a particular developmental process may be determined very largely by the choice of variable which is involved in the establishment of a scale of physiological age. Furthermore, a single dimension will usually only be a satisfactory indicator of physiological age over a limited range; the length of an internode, for instance, reaches a final value and ceases to register. It follows that a quantity which is an excellent measure of physiological age for one investigation may be quite useless for another.

It is therefore natural to inquire whether there may not be some measure of physiological age which is more fundamental or more widely applicable than others, and worthy of being adopted as a standard to which all observations may ultimately be referred. It has been very generally supposed that in studies on developing shoots the regular succession of leaves at the apex would afford the basis for such a scale, and the interval between the development of one leaf and that of the next has come to be known as a plastochrone.

Unfortunately the application of the plastochrone concept to purposes of exact measurement is attended with formidable difficulties, the existence of which has commonly been overlooked. As usually understood, a plastochrone is the time-interval which elapses between a particular stage in the development of one leaf and a corresponding instant in the development of the next leaf. This mode of definition requires the selection of some criterion by which to recognize the 'particular stage of development'. It has often been tacitly assumed that the magnitude of the plastochrone is independent of the choice of criterion, but there is no logical basis for this belief, and many observations tend to discredit it. An even more serious difficulty arises from the fact that the definition depends on the recognition of isolated events, so that the scale of plastochrones is inherently discontinuous. The changes which occur in a shoot during the course of a single plastochrone are often very extensive, so that for many purposes the plastochrone is an inconveniently large unit. Before the plastochrone scale can be widely used in quantitative work it will be necessary to find a method for the estimation of fractional values. The manner in which this problem arises may be shown by a fictitious example. Suppose that in a particular batch of material it is found that plants in which the fifth leaf has just reached a standard degree of unrolling have a fifth internode of average length 30 mm., while in plants in which the sixth leaf is at the same stage the fifth internode has an average length of 80 mm. Then, the definition of a plastochrone once established, it may be said that during the sixth plastochrone of growth the fifth internode elongates at an average rate of 50 mm. per plastochrone. This is a clear and unambiguous statement of morphological fact. But what reply is to be given to an inquirer who wishes to know how the growth rate of the fifth internode, expressed in millimetres per plastochrone, varies during the course of the sixth plastochrone of the plant's age? Unless means can be found for the measurement of fractional plastochrone intervals, this question must remain unanswered. The question cannot, however, be dismissed as a meaningless one. There is nothing unreasonable in its form, and our inability to find an answer is no justification for concluding that no answer exists.

It may be suggested that the measurement of fractional plastochrone intervals could be accomplished by obtaining a conversion factor expressing the duration of a plastochrone in ordinary chronological units. This procedure, however, besides requiring perfect standardization of genetic constitution and environmental conditions, would only be valid if the conversion factor were independent of age, a condition which seems unlikely to occur in any real case.

The establishment of a plastochrone scale which will allow of fractional measurements is mathematically equivalent to the establishment of a scale based not on observations of a series of widely spaced isolated events but on the study of infinitely divisible processes; in other words, the plastochrone must be defined in terms of continuously varying quantities like internode length or leaf area.

An internode is one plastochrone older than the one above, and one plastochrone younger than the one below; this statement is in itself a definition of the plastochrone, and does not depend on the selection of any particular criterion by which the end of the plastochrone is to be recognized. Let X be any measurable property of the internode. Then if simultaneous measurements of X were made for all the internodes of a single shoot, a graph of X against the plastochrone age of the internode, which will be denoted by t, could be plotted by spacing the X-values obtained from successive internodes at unit intervals along the axis of physiological age. But this procedure would be valid only if every internode reproduced the behaviour of the one below; otherwise the graph obtained would be a resultant curve, representing a summation of the changes due to increasing age with the inherent differences between the internodes. The subsequent discussion applies only to cases in which all internodes behave similarly in respect of the dimension X.

It might appear that by running a smooth curve through all the points a graph of X against t could be prepared from which the plastochrone age of any internode whatever could be obtained by measuring X and reading off the corresponding value of t. In this way fractional ages could be determined without difficulty. The method is theoretically sound, but two causes combine to render it utterly useless in all real situations. In the first place the points, being separated by time-intervals of one plastochrone, are too widely spaced for the satisfactory construction of the smooth curve required. Secondly, as the graph is based on measurements of only a single shoot there is no possibility of controlling sampling errors. In order to overcome these obstacles it is necessary to find a method for combining in one graph observations taken from internodes which are not products of the same apical meristem. Furthermore, the method must not require any knowledge of the age-differences between such internodes, since these differences will in general be fractional and so cannot be directly measured. It follows that the desired combination must be effected by the use of equations having no dimension of time. As the only other variables involved are measurements of X, the equations required will necessarily have the dimensions of X.

Consider the following procedure. It must always be possible to obtain an empirical equation of the form

$$X_{n+1} = f(X_n), \tag{1}$$

where X_n and X_{n+1} represent simultaneous X-values of two successive internodes. Generally speaking there will be a different equation of this type for every value of n, in which case the analysis can be carried no farther. But if all internodes behave alike, the same equation will apply to all, and we may write:

$$X_t = f(X_{t+1}), \tag{2}$$

where X_t and X_{t+1} represent X-values of the same internode measured one plastochrone apart. We have now to solve the purely mathematical problem of deducing from (2) an equation of the form

$$X_t = f'(t). \tag{3}$$

The method is governed by some important general principles, which are almost self-evident. Every equation in class (1) is uniquely related to one in class (3). If there are N constants in a class (1) equation the related equation of class (3) will have $(N+1)$ constants. One of these will be the value of X_t when $t = 0$, and must be arbitrarily selected, serving merely to fix the zero point of the plastochrone scale. The remaining constants of the class (3) equation will be derived by calculation from those of the corresponding equation in class (1).

No great difficulty is likely to be encountered in deriving from any given equation of class (1) the corresponding equation in class (3), but a considerable amount of working may be involved, and the connexion between the two equations will not usually be apparent upon mere inspection. It has therefore been thought desirable to place on record the results so far obtained, and an appendix to this paper gives the class (3) equations corresponding to linear and allometric relationships in class (1).

The first stage in the investigation of the linear density measurements is the preparation of a series of graphs of W_{n+1} against W_n, smoothing out the irregularities of the data by combining observations in sets of five, as explained in connexion with the study of xylem (Dormer, 1950, p. 426). When this is done it is found that all the graphs are nearly identical. Small differences, almost undetectable in a drawing of any reasonable size, can be demonstrated by close examination, and will be discussed in a later part of this paper, but no great loss of precision will result if the data are treated in the first place as though the form of the graph of W_{n+1} against W_n were independent of n. In Fig. 1 each point represents the means of five pairs of simultaneous observations of W_n and W_{n+1}. In grouping the data observations for different values of n have been kept separate, but in plotting the graph no attempt has been made to distinguish between the sets of points for different pairs of internodes. Instead of undertaking regression calculations, we shall use the equation

$$W_{n+1} = 0{\cdot}8\ W_n, \tag{4}$$

the graph of which is shown, and which fits the data well enough for the

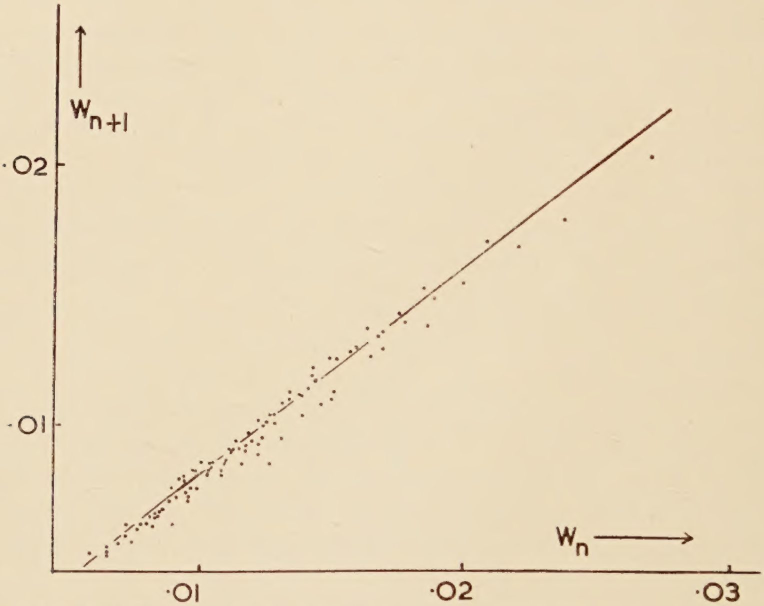

Fig. 1. Graph of W_{n+1} against W_n. Each point represents five pairs of observations. The straight line represents the equation $W_{n+1} = 0{\cdot}8\ W_n$.

purposes of the present paper. As equation (4) is very nearly true for all values of n within the observed range, we may write:

$$W_t = 0{\cdot}8\ W_{t+1}, \tag{5}$$

or

$$W_{t+1} = 1{\cdot}25\ W_t,$$

and this relationship may be repeated indefinitely, thus:

$$W_t = 1{\cdot}25\ W_{t-1} = 1{\cdot}25^2\ W_{t-2} = 1{\cdot}25^3\ W_{t-3} \ldots = 1{\cdot}25^t\ W_0.$$

This result, which might have been obtained by referring to the equations given in the Appendix, is in accordance with the general principles laid down above. An equation has been developed which connects the linear density of an internode with its plastochrone age, and an extra constant has appeared in the process. This constant, W_0, represents the linear density of an internode of zero age, and may be assigned any value, serving merely to fix the zero of the age-scale. It would be convenient to eliminate W_0 by putting

$W_0 = 1$. As the linear density of an internode never attains 1 g. cm.$^{-1}$, this would have the effect of making the ages of all real internodes negative. To avoid this it will be best to reduce to milligrammes, thus:

$$1{,}000\ W_t = 1{\cdot}25^t. \qquad (6)$$

A plastochrone scale has now been established on which the age of an internode is to be determined by multiplying its linear density by 1,000 and taking the logarithm to the base 1·25 of the product. There is no difficulty in dealing with fractional values, and were it not for the approximations involved in the calculation of the scale the average age of a group of internodes could be determined to any required degree of accuracy, although individual observations must always be subject to random errors.

As the records of linear density are accompanied by estimates of plastochrone age obtained by observation of the unfolding of the leaves, it is possible to make a comparison between the scale of plastochrones derived by calculation from the linear density measurements and that based on the direct observation of the developing leaves. The two scales have, of course, different zero points, so that it will be necessary to find by trial a point on one scale equivalent to a point on the other. It will then be possible to see whether the intervals of the two scales are of the same magnitude. For this purpose it is necessary to plot, for each value of n, a graph of linear density against the plastochrone age (from leaf observations) of the internode, and then to superimpose on these graphs the curve of linear density against plastochrone age derived from equation (6), sliding the curve to right or left until the best fit is obtained.

The results of this operation are shown in Fig. 2, where the graph of equation (6) is represented by the broken line. The two plastochrone scales are shown at the foot of the graph; they are fitted together on the basis that zero age, from leaf observations, is equal to an age of 7·77 plastochrones, from linear density measurements. Whereas the calculated curve represents with reasonable accuracy the earlier stages in the growth of the lower internodes, it is clear that the later increases in linear density are much smaller than equation (6) would lead one to expect. That is to say, in the later stages of plumular growth the development of the stem is retarded relative to the processes of leaf production. The numbered arrows in Fig. 2 indicate the points at which the graphs of linear density for the different internodes cease to follow the calculated curve, and it will be seen that they are simultaneous points, since the sum of the serial number of the internode and the plastochrone age at which its development departs from the calculated curve is constant, and this sum is equal to the plastochrone age of the plant at the time. In plants up to the age of 8 plastochrones (from leaf observations) the development of the stem, as measured by the increase in linear density, keeps pace with the development of the leaves. After this point in the life-cycle has been passed, the development of the stem lags behind that of the leaves, though the various internodes continue to keep in step with each other. On

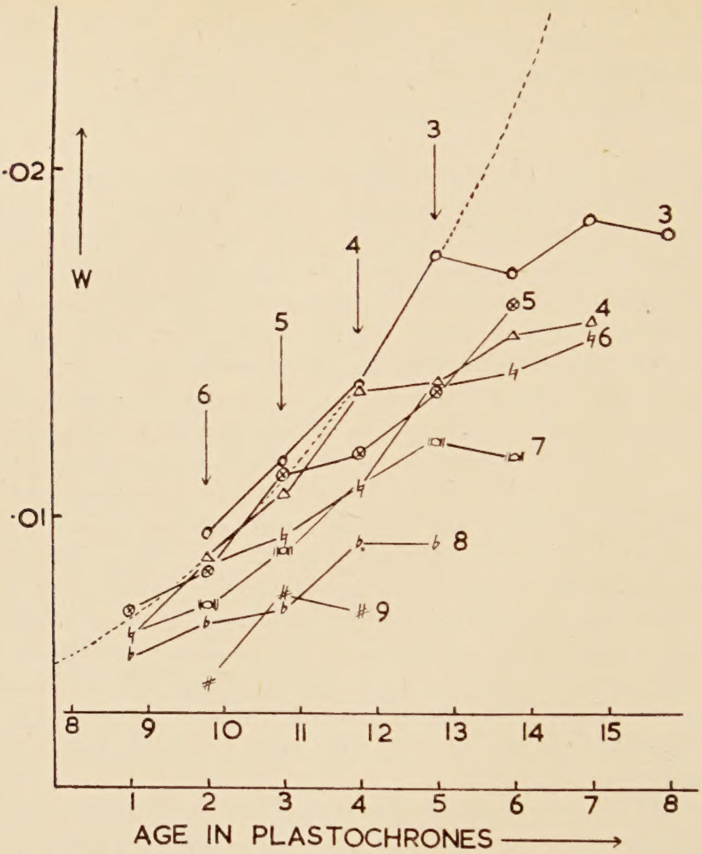

FIG. 2. Graphs of *W* against the plastochrone age of the internode. Lower plastochrone scale is derived from observations on the unfolding of leaves, upper scale is that given by equation (6), the graph of which is shown as a broken line.

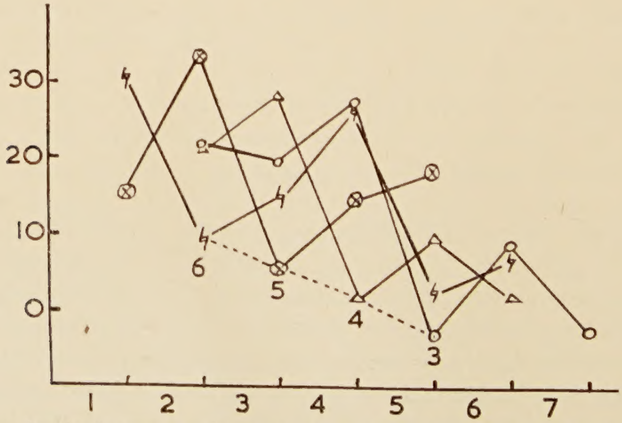

FIG. 3. Linear density increments (percentage per plastochrone) plotted against the plastochrone age of the internode.

the right-hand side of Fig. 2 the graphs are arranged roughly in serial order, owing to the earlier incidence of the change in the ontogeny of the higher internodes. The data may also be examined in another way. In Fig. 3 there

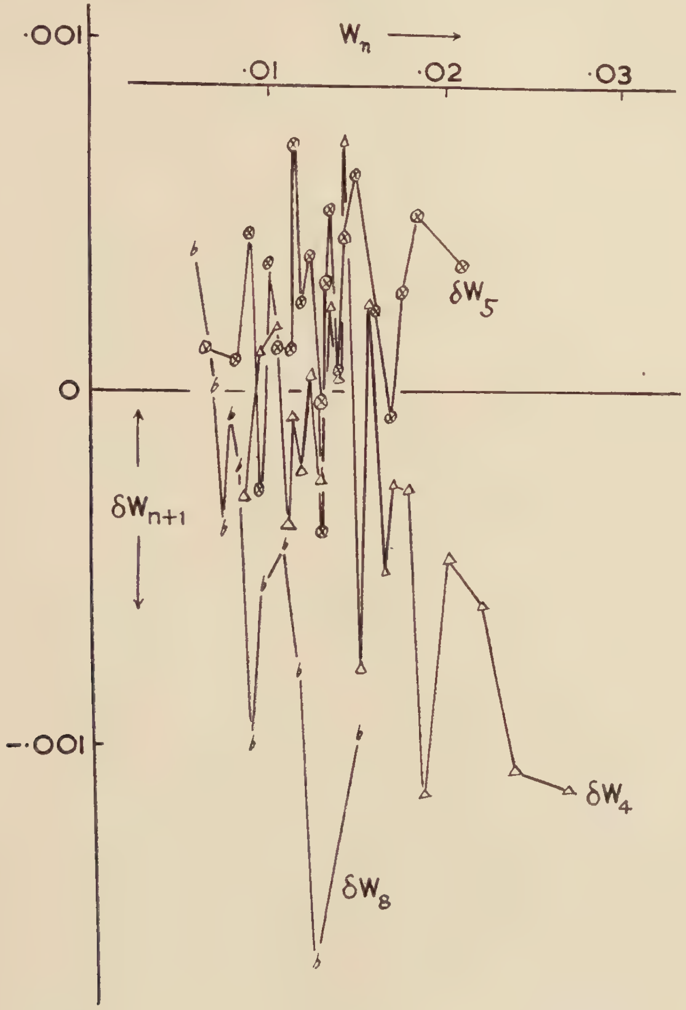

FIG. 4. Graphs of δW_{n+1} against W_n for $n = 3$, 4, and 7.

is shown for each internode the percentage increase in its linear density for each plastochrone of its growth. It will be seen that the graph for each internode shows a sudden drop, and that the minima which are reached are simultaneous points, occurring in plants aged 8–9 plastochrones. It is clear also that the minimum growth-rate is least in the lowest internodes. It is possible that in the third internode at this time there is actually a decrease in linear density, though the evidence on this point cannot be regarded as conclusive.

It has already been stated that there are in fact slight differences in behaviour between the different internodes, but that these differences are too small to be clearly shown in a graph of W_{n+1} against W_n plotted in the usual way. In order to study these differences it is necessary to find a means of magnifying them. If we rewrite equation (4) in the more accurate form

$$W_{n+1} = 0{\cdot}8\, W_n + \delta W_{n+1}, \tag{7}$$

each point on Fig. 1 will yield an estimate of δW, and we can plot a series of graphs of δW_{n+1} against W_n. If all these graphs were plotted on the same sheet an extremely confused and intricate figure would be produced. It has been thought sufficient to show in Fig. 4 the graphs for $n = 3$, 4, and 7, representing separately (Fig. 5) the variation with n of the mean value of δW_n. From this work it is clear that the value of W_{n+1} for a given value of W_n is greatest when $(n+1) = 5$, and that the differences between internodes are most pronounced in the later stages of development.

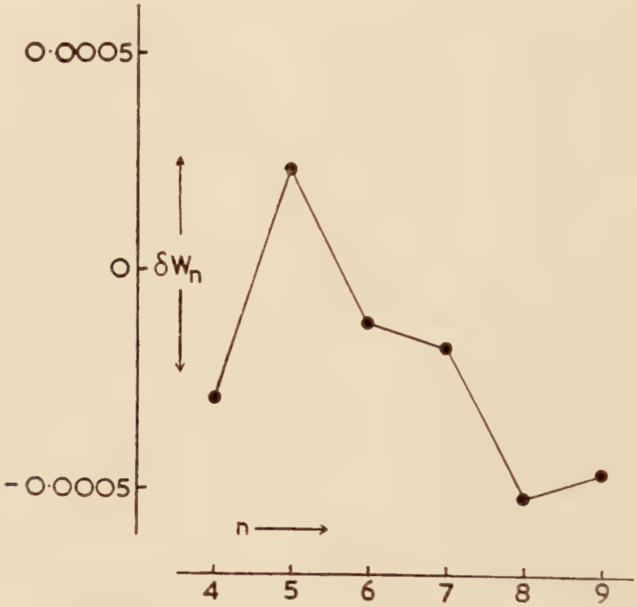

FIG. 5. Mean values of δW_n plotted against n.

DISCUSSION

As a result of the observations described in this paper, it now seems unlikely that the plastochrone concept can occupy any permanent place in quantitative studies of the developing bean plumule. Were it possible to establish a continuous plastochrone scale related in a simple manner to the succession of leaves, there would be every reason for expressing all estimates of physiological age in terms of plastochrones. It has been shown, however, that a continuous scale must be based on measurements of some quantity which satisfies the condition that the relationship between simultaneous measurements in two successive internodes is independent of serial number, that the only known quantity which even approximately satisfies this condition is linear density, and that the scale derived from the linear density measurements is incompatible with any conceivable system of observations on leaf succession. Under the circumstances, it is only reasonable to suspect that in the bean shoot the plastochrone, as a fundamental, universally applicable unit of measurement, is a myth. It may still be convenient, for particular purposes, to employ 'plastochrone' scales, but the selection of such a scale, in preference to some other form of expression, must be regarded as a matter of expediency rather than as an intrinsically desirable procedure possessing special theoretical significance. Until the contrary is proved, it must be taken for granted that 'plastochrone' scales defined in different ways will have intervals of different magnitudes.

Quite apart from their accidental importance in connexion with theoretical problems of physiological age measurement, the observations on linear density are of some interest for their own sake. The most outstanding feature is the sudden change in the mode of distribution of solid material in the plant which occurs between the ages of 8 and 9 'plastochrones'. In the early stages of growth every addition to the solid substance of the plumule is divided according to a fixed and relatively simple mathematical rule between the leaves and the various internodes of the stem. A sudden crisis then occurs, after which a new system of division comes into force. The stem now receives a much smaller share than would have been due to it under the previous régime, though the mechanism governing the allocation of the stem total among the individual internodes apparently remains unchanged. The simplest hypothesis appears to be that the change is due to the exhaustion of the cotyledonary reserves, the mechanism governing the distribution of photosynthetic products being different from that which controls the movement of reserve foods. Irrespective of the truth of this supposition, the change in the mode of distribution of material implies a relationship between the position of an internode and its nutrition. An internode at the base of the shoot receives in the first place a relatively lavish supply of material and reaches an advanced condition of maturity before this supply is suddenly and drastically reduced. An internode high up in the stem, on the other hand, experiences the cut while still very young and is afterwards dependent on what is, by comparison, a meagre trickle of substance.

The graph of W_{n-1} against W_n undergoes, as n increases, a succession of changes of a kind already familiar from previous investigations in this series. Altogether four distinct and apparently independent mathematical relationships are now known to respond in essentially the same manner to an increase in serial number. In each case the effect appears as a reversible displacement of the characteristic graph of the relationship considered. When the length of a petiole is plotted against the length of the corresponding internode the point of reversal is found to lie in the fifth internode (Dormer and Plack, 1951, Fig. 8); the graphs of linear density also show a reversal at the fifth internode (present paper, Fig. 5). In respect of the relationship between the vascular development of a leaf-trace bundle and that of the internode in which it runs, the point of reversal cannot be so exactly located, but it certainly lies either in the fifth internode or in the fourth (Dormer, 1950, Figs. 4–6). Similarly, the relationship between the vascular development of an internode and the unfolding of the associated leaf has a point of reversal either in the fifth internode or in the sixth (Dormer, 1950, Fig. 2).

It is impossible to overlook the unique position which is occupied in the foregoing discussion by the fifth plumular internode. It seems likely that this internode is of special significance in the life of the shoot, and that it is distinguished from those above and below by possessing the following combination of attributes:

1. Maximum linear density for any given linear density of the internode below.
2. Maximum length for any given length of its own petiole.
3. Minimum vascular development at the time when its leaf unfolds.
4. Maximum vascular development of the median trace bundle of its leaf for any given degree of vascular development of the other bundles of the stem.

Unless the concentration of all these peculiarities in the same internode is to be dismissed as a meaningless coincidence, it is necessary to suppose that they are all manifestations of the action of some unknown factor which is distributed in the shoot so as to have a maximum (or minimum) intensity in the fifth internode, and which exerts an influence on all the different developmental processes so far examined. If such a mechanism exists, then it is to be expected that the fifth internode will occupy an equally prominent place in future investigations of other variables.

We have now to consider to what extent observed differences in linear density can reasonably be attributed to known differences in the degree of vascularization. In certain respects the two measurements obviously behave similarly. The dry-weight observations, like the xylem cell-counts, indicate that the early development of an internode is almost independent of its position in the stem, the differences between the internodes becoming apparent only in the later stages of growth. It seems likely also that the sudden reduction in the rate of increase of the linear density of the stem may be merely a consequence of the retardation of xylem development which, as shown in a previous paper (Dormer, 1950, p. 433), affects the upper internodes at about the same time. No simultaneous retardation of the growth of the xylem in the lower internodes has been demonstrated by direct observation; the linear density measurements in this instance may throw some light on a phenomenon which is outside the range of the cell-counting technique.

It is clearly desirable to ascertain by calculation whether the observed differences in linear density are such as might be expected to occur as a result of the known differences in numbers of xylem cells. No very intimate acquaintance with the problem is required to show the futility, in the present state of our knowledge, of any attempt to secure accuracy in such a calculation. Using crude approximations, however, it can easily be shown that the linear density differences are of about the order of magnitude which would be expected if they were entirely due to the differences in xylem structure. In order to relate the calculation to familiar anatomical realities, let us consider the development of the xylem in a transverse section of thickness 10 μ. It appears from the graphs that the dry weight of such a section in a moderately mature condition might be about 10^{-5} g. Let us suppose that any xylem cell which develops subsequently is square in section, and 15 μ across, with a wall 2 μ thick. These dimensions are quite arbitrary, but they are at least not hopelessly unrealistic. A cell with the specified dimensions adds to a 10 μ section slightly more than 10^{-9} cm.^{3} of lignocellulose wall. The specific gravity of the wall is unknown, but it must be greater than unity, and something has in any case to be allowed for cell contents and for the occasional presence in the section of a transverse wall. We may therefore estimate the dry weight of the piece of the cell which lies in a 10 μ section as 2×10^{-9} g. From Fig. 5, a reasonable figure for the range of variation of δW is, in round figures, 5×10^{-4} g. cm.$^{-1}$, representing in the section a weight of 5×10^{-7} g. Dividing this difference by the estimated addition of weight due to the presence of a single extra xylem cell, we obtain 250 as the number of cells by which any two internodes might, on the basis of the linear density measurements, be expected to differ. It seems from previous work (Dormer, 1950, Fig. 3) that the differences which actually occur are of the order of 150–200, so that we shall commit no obvious absurdity in concluding that the observed differences in linear density are mainly the result of differences in vascular structure.

In one instance, however, there seems to be a discrepancy between the observations on linear density and those on the number of xylem cells. Taking each internode at a given stage of development of the one below, the fifth internode is heavier than the fourth (Fig. 5), but contains a smaller number of xylem cells (Dormer, 1950, Fig. 3). Should these observations be confirmed, it will be necessary to conclude either that the xylem cells of the fifth internode are heavier than those of the fourth (which seems unlikely, since there is no obvious histological difference between the xylem tissues of the two internodes) or that in the fifth internode a greater proportion of the total dry weight is present in the form of extra-xylary tissues. Unfortunately no method appears to be available by which the dry weights of the various tissues in a transverse section can be separately determined.

SUMMARY

As an alternative to the counting of xylem cells, the development of the bean shoot has been studied by cutting a piece out of each internode and determining the dry weight per unit length of stem.

This quantity, which is called the linear density of the internode, is in most respects closely correlated with the degree of vascularization as measured by cell-counts, but in the fourth and fifth internodes of the plumule there is a discrepancy which may indicate that the distribution of dry weight among the tissues in a transverse section is different in different internodes.

It is shown that the linear density measurements can be used in the construction of a scale of physiological age, based upon a new principle. The results suggest that the concept of a plastochrone as a fundamental unit of measurement is an illusion, at least in the bean shoot.

During the unfolding of the ninth leaf there is a sudden change in the mode of distribution of the total dry-weight increment, resulting in a drastic reduction in the growth rate of the stem without any corresponding retardation of the processes of leaf development. The structure of an internode largely depends on the age which it has reached at the time when this change takes place, which is in turn a function of the serial number of the internode.

Taken in conjunction with the results of previous work, the observations on dry weight strongly suggest that many of the processes occurring in a growing shoot are influenced by some unknown factor which is so distributed as to have a maximum (or minimum) intensity in the fifth internode.

APPENDIX

Let X be any measurable property of an internode (or leaf), X_n and X_{n+1} being the values obtained from simultaneous observations on any two consecutive internodes. Then provided that the relationship between X_{n+1} and X_n is the same for all values of n, it is possible to obtain an equation expressing X_t, the X-value of an internode aged t plastochrones, in terms of t, the plastochrone age. The solutions for two important cases are given below.

CASE I. Linear relationship between X_{n+1} and X_n.

Let $$X_{n+1} = mX_n + C \tag{8}$$

where m and C are constants.

Then $$X_t = b^t X_0 - d(b^t - 1) \tag{9}$$

where $b = 1/m$, $d = C/(1-m)$, and X_0 is the value of X_t when $t = 0$.

CASE II. Allometric relationship between X_{n+1} and X_n.

Let $$\log X_{n+1} = m \log X_n + C, \tag{10}$$

where m and C are constants.

Then $$X_t = k\, e^{-e^{a-bt}}, \tag{11}$$

which is the equation of a Gompertz curve (Winsor, 1932), the constants being obtained as follows:

$$b = \log m, \quad k = \operatorname{antilog} C/(1-m), \quad \text{and } a = \log\log k/X_0,$$

X_0 being the value of X in an internode of zero age.

NOTE. X_0 may be assigned any convenient value which is a possible value of X for real finite values of t. If this condition is not satisfied absurd results will occur. It will generally be advisable to make $X_0 = 1$; if unity is not a possible value of X it can always be made so by altering the units in which X is measured.

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Studies on the Growth of Basidiomycetes in Soil

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With Plate XV and two Figures in the Text

SUMMARY

Fructifications of basidiomycetes have been collected at Lakenheath Warren, and thirty-nine species have been obtained from five grassland soils. The different soils have been found to have different species growing upon them and most species occur on the alkaline-slightly acid grasslands. Highly acid grassland at Lakenheath has a poor fungus flora.

Studies have been made of the regions in the soil in which the mycelia of certain of these basidiomycetes occur, and attempts have been made to isolate these fungi from soil. Some species have been found to inhabit the litter zone rather than the mineral layers of the soil. A few form extensive rhizomorph systems which may penetrate deeply into the soil. A few species of ring fungi, such as *Marasmius oreades*, *Psalliota arvensis*, and *Tricholoma nudum*, have been found to possess a well-defined mycelial zone in the soil, from which the fungus could be isolated.

Profile studies have shown that the mycelial zones of these species contain a restricted population of microfungi, both fewer species and fewer colonies, as compared with the normal soil around. Ascomycetes, such as *Arachniotus*, *Chaetomium*, *Gymnoascus*, and *Penicillium*, have been isolated more frequently from mycelial zones than from normal soil.

INTRODUCTION

ALTHOUGH the fructifications of basidiomycetes have been extensively collected and studied over many years, there is surprisingly little information on the mycelial growth habit and ecology of these fungi in the soil. It has become recognized that different plant communities, such as beech or pine woods, have different fungal floras, and literature is full of references to the habitats and seasons of certain species. A few, more comprehensive, ecological studies have been made. They show that fungal mycelia may exist in the soil for several years, that they may not fruit every year (depending on environmental and other conditions), and that fungal communities are governed by the soil type as well as the vegetation of an area. Bridge Cooke (1948) gives a good review of the literature on fungus sociology and ecology, especially that of the German workers.

There appears even less published work on the mycelial growth habit of basidiomycetes in the soil. Some studies, such as that by Shantz and Piemeisel (1917), have been made on the fungi which form fairy rings. These

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studies have mainly been concerned with the type of ring formed and its effect on the vegetation. Soil microbiologists have not studied basidiomycetes other than to remark on the scarcity of colonies, directly or indirectly referable to these fungi, which can be obtained on soil isolation plates. Thus Waksman (1927) remarks 'our methods are not sufficiently developed as yet to allow the direct isolation of certain organisms like the basidiomycetes (from soil)'. From their study of soil fungi in Manitoba, Bisby et al. (1935) were led to remark 'the isolation technique ordinarily used by the soil microbiologist yields little information on the flora of Hymenomycetes and Gasteromycetes known to be abundant in the soil'. It has been suggested that basidiomycetes do not grow on isolation plates, particularly in competition with fast-growing microfungi. However, little experimental work has been done on this problem.

While studying the microfungi of five grassland soils at Lakenheath Warren, Suffolk, the writer took the opportunity of investigating some of these problems concerning the growth of basidiomycetes in soil. Two main lines of research have been followed. The first consisted of a study of the basidiomycetes occurring on Lakenheath Warren, and fructifications were gathered on the many visits the writer made to that area from the spring of 1947 to the autumn of 1949. The second was the more general problem of the growth of basidiomycetes in soil and whether it is possible to isolate them by a plating technique. Soil plates (Warcup, 1950) had been found to isolate a wide range of soil fungi, and it was thought that by their use basidiomycetes might be isolated. As random soil sampling has yielded few cultures of basidiomycetes in past investigations, a study of the mycelial growth of these fungi in the soil was made to investigate whether this was a factor influencing their isolation from soil.

A. Fungi obtained from Lakenheath Warren

The fungi recorded in this list were collected from five of the series of grassheath soils distinguished at Lakenheath Warren by Watt (1936, 1940). These soils, although all dry and sandy, vary considerably in their pH and calcium carbonate values. Grasslands *A* and *B* are alkaline, grassland *C* varies about neutrality, and grasslands *D* and *E* are highly acid. Fungi were collected only from the undisturbed grassheath, and no specimens were taken from the areas of *Carex arenaria*, bracken, heather, or blown sand, which also occur on the warren. The list probably does not contain all the species which grow on these grassland soils, as sampling at fortnightly to monthly intervals allows the possibility of some species fruiting and dying between visits. However, during the autumn of 1948, when most of the fungi were collected, visits were made once a week. The differences between the number of species recorded in 1947 and 1949, poor fungus years, and those in 1948, a good one, make it seem highly probable that other fungi are present which did not fruit in any of those years. Lange (1948), after a three-year study of the agarics of Maglemose, considered that that period was not sufficient to record all the fungi present in the region.

List of species

ASCOMYCETES

Cordyceps militaris (Linn. ex Fr.) Link
Geoglossum cookeanum Nannf.
Peziza rutilans Fr.

BASIDIOMYCETES

Agaricales (including *Clavariaceae*)

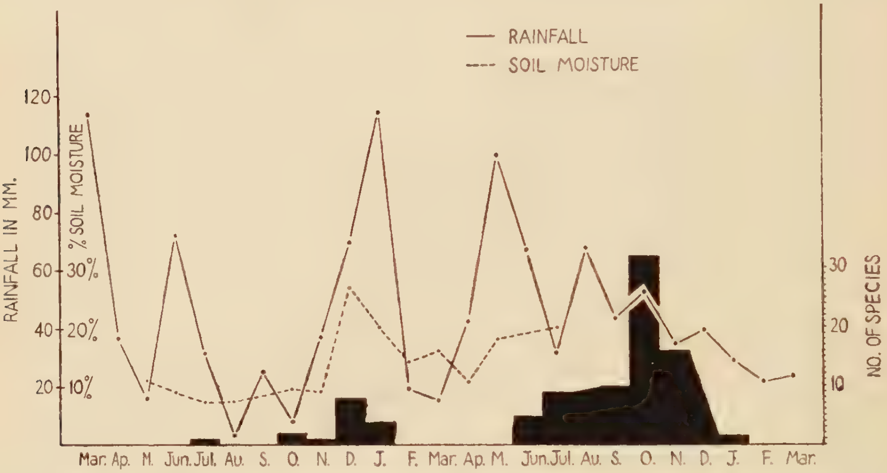
Clavaria argillacea Pers. ex Fr.
C. corniculata Schaeff. ex Fr.
Clitocybe cyathiformis (Bull.) Fr.
C. ditopus Fr.
C. rivulosa (Pers. ex Fr.) Quél.
Crinipellis stipitarius (Fr.) Pat.
Entoloma sericeum (Bull. ex Fr.) Quél.
Galera hypnorum (Batsch) Fr.
G. rubiginosa (Pers. ex Fr.) Gillet
Hygrophorus miniatus Fr.
H. nigrescens Quél.
H. reai Maire
H. subradiatus (Schum. ex Fr.) Fr.
H. virgineus (Wulf. ex Fr.) Fr.
Lepiota procera (Scop. ex Fr.) S. E. Gray
Leptonia incana (Fr.) Gill.
Marasmius oreades (Bolt. ex Fr.) Fr.
Mycena aetites Fr.
M. avenacea Fr.
M. filopes (Bull. ex Fr.) Quél. non Kühner
M. vitilis Fr. non Kühner
Nolanea papillata Bres.
Omphalia rustica (Fr.) Quél.
Panaeolus campanulatus (Linn.) Fr.
Psalliota arvensis (Schaeff. ex Fr.) Quél.
Psilocybe bullacea (Bull.) Fr.
P. atrorufa (Schaeff. ex Fr.) Quél.
Ripartites tricholoma (A. & S. ex Fr.) Karst.
Stropharia coronilla (Bull. ex Fr.) Quél.
Tricholoma irinum (Fr.) Quél.
T. melaleucum (Pers. ex Fr.) Quél.
T. nudum (Bull. ex Fr.) Quél.
Tubaria pellucida (Bull. ex Fr.) Gill.

Lycoperdales

Lycoperdon caelatum Bull. ex. DC.
L. perlatum Pers.
L. spadiceum Pers.

Seasonal occurrence

The season and its weather conditions were found to influence the occurrence of fungi on Lakenheath Warren. Most species fruited in autumn, although there was a great difference in time of fruiting and the number of species recorded in different years. Text-fig. 1 shows the number of species found each month over a period of 2 years and gives rainfall and percentage soil moisture data. The rainfall figures are for Santon Downham, about 3 miles from Lakenheath Warren, and were obtained from the official Meteorological Reports published by H.M. Stationery Office. Soil moisture figures are for grassland *B* and were obtained from samples collected at 1 in. from the surface.



TEXT-FIG. 1. Influence of rainfall and soil moisture on the number of species of fungi fruiting each month.

The influence of low summer rainfall and low soil moisture on the number of species found in autumn is easily seen. Lakenheath Warren also seems rather unusual in the long period, February to May, during which no sporophores were found. Wilkins and Patrick (1940) in their investigations of the fungi of three grassland sites found a few species fruiting at all seasons of the year. As soil moisture and rainfall are often high during February to May, it seems there are other factors involved in the fruiting of such fungi, as has been suggested by Grainger (1946).

Occurrence of fungi on the five soils

The occurrence of the sporophores of fungi on the five soils during 1947–9 is recorded in Table I. The areas from which the fungi were collected are not comparable in size, grasslands *A* and *C* being smaller than the rest. *Hygrophorus nigrescens* was the most widely distributed fungus found on the grassland soils of Lakenheath Warren. Most species were recorded from soils *B* and *C*, and the more alkaline and very shallow soil *A* and the highly acid soils

D and *E* had much poorer fungal floras. The poor flora of the *E* soil forms a contrast to the flora from a similar soil under a stand of pines at Brandon Park, about 2 miles away. Here the soil supports a considerable flora, with common species such as:

Amanita gemmata (Fr.) Gill.
A. rubescens (Pers. ex Fr.) S.F. Gray
Boletus luteus L. ex Fr.
B. scaber Bull. ex Fr.
Hygrophorus hypothejus Fr.
Laccaria laccata (Scop. ex Fr.) Cke.
Lactarius plumbeus (Bull. ex Fr.) S.F. Gray
Russula ochroleuca (Pers. ex Fr.) Fr.
Thelephora terrestris Ehrh. ex Fr.

TABLE I

Occurrence of Fungus Fructifications on Five Grassland Soils

Fungus	Soil type				
	A	*B*	*C*	*D*	*E*
Hygrophorus miniatus	+				
Omphalia rustica	+				
Tubaria pellucida	+				
Leptonia incana		+			
Lycoperdon perlatum		+			
Hygrophorus reai		+			
H. subradiatus		+			
Psalliota arvensis		+			
Ripartites tricholoma		+			
Tricholoma irinum		+			
Crinipellis stipitarius	+	+	+		
Stropharia coronilla	+	+	+		
Clitocybe rivulosa		+	+		
Entoloma sericeum		+	+		
Galera hypnorum		+	+		
G. rubiginosa		+	+		
Lycoperdon spadiceum		+	+		
Marasmius oreades		+	+		
Mycena aetites		+	+		
Panaeolus campanulatus		+	+		
Tricholoma melaleucum		+	+		
Clitocybe cyathiformis			+		
C. ditopus			+		
Nolanea papillata			+		
Hygrophorus nigrescens	+	+	+	+	
H. virgineus		+	+	+	
Lepiota procera		+	+	+	
Lycoperdon caelatum		+	+	+	
Mycena avenacea		+	+	+	
Tricholoma nudum		+		+	
Mycena filopes				+	+
M. vitilis				+	+
Psilocybe bullacea					+
Ps. atrorufa					+
pH of soil (av.)	8·0	7·6	6·4	4·4	3·8

How much the distribution of fungi on Lakenheath Warren is governed by plant cover rather than soil type is not known. Wilkins and Patrick (1939) reported *Tricholoma melaleucum* as being associated with *Carex glauca* on the soils they studied, but *Carex glauca* is not present on Lakenheath Warren. In this study *Clitocybe rivulosa* seemed often associated with *Carex* spp. and the mycelium of the fungus could be found on *Carex* roots, but whether the association is constant is not known. *Crinipellis stipitarius* was always found on dead grass stems and leaves, mainly of *Festuca ovina*. The sporophores of *Stropharia coronilla* were always found associated with grass plants. Masses of powdery white mycelium were present at the base of the leaf sheaths but did not appear to harm young tissues. Mycelium could be found in this position in *Festuca* irrespective of the presence of sporophores and could be traced along the rhizomes of the grass. *Clavaria argillacea* seemed to be associated with *Calluna vulgaris*. This fungus was common on the heathy areas adjacent to grasslands *D* and *E*. On the only occasion when it was seen on grassland *E* the sporophores were grouped in a ring around a small bush of heather.

B. MYCELIAL GROWTH HABIT AND THE ISOLATION OF BASIDIOMYCETES FROM SOIL

In autumn when sporophores became plentiful on Lakenheath Warren, some of the more common fungi were collected to examine the location of their mycelium in soil. The excavation of mycelium under sporophores was carried out in the field, or blocks of soil with sporophores were brought back to the laboratory. Soil blocks kept in closed containers often developed profuse mycelium either from the soil or from litter near the sporophores. The basidiomycete could occasionally be isolated directly from this mycelium. Soil plates also were prepared from soil collected beneath clumps of sporophores. Cultures were prepared from young gill tissue of the sporophores of many basidiomycetes for comparison with cultures obtained from litter or soil.

It was soon evident, even from the few species investigated, that different basidiomycetes live in different regions of the soil, and that a knowledge of the mycelial growth habit of a fungus is important in any attempt to isolate it from soil. On the basis of the position of the mycelium in the soil, basidiomycetes may be divided into several groups, three of which are recorded in this paper. It should be noted that these groups do not include more than a part of the fungi growing on Lakenheath Warren, and that some species (*Hygrophorus nigrescens* and *Clavaria* spp.) proved intractable by the methods used in this study and little information has been gained about their mycelial growth.

Some species were found to inhabit the litter zone rather than the mineral layers of the soil. An example is *Crinipellis stipitarius*, which was found growing on dead grass leaves and stems. It was often isolated from such a habitat whether or not the fructifications of the fungus were present. In no case was the fungus recorded from soil. As several other kinds of mycelium with clamp connexions were isolated from plant debris in the litter zone, it seems probable that several basidiomycetes live mainly, if not exclusively, in the litter.

A few species were found to possess extensive rhizomorph systems in the soil. An interesting example is *Lycoperdon caelatum*, the fruit bodies of which were common on Lakenheath Warren. They were solitary and sporadic or in lines which were possibly parts of large rings. The sporophores were always found attached to a stout rhizomorph, which went almost vertically into the soil for 2–4 in., and then connected with a branching rhizomorph system of much smaller, shining, white strands. These small strands were often found to penetrate to a depth of 12–14 in. Pieces of rhizomorph, washed and plated, were found to grow on several media, though microfungi were often present and overgrew the basidiomycete.

Three fungi, *Marasmius oreades*, *Psalliota arvensis*, and *Tricholoma nudum*, were found to have definite mycelial zones in the soil from which they could be isolated. They are also interesting in that very few species of microfungi were isolated from these mycelial zones. Extensive experiments were carried out with these species and are presented in detail for each fungus.

Marasmius oreades

The phenomenon of a restricted microflora, in what may be called the 'mycelial zone' of a basidiomycete, was first noted with this species. *M. oreades* is common on grasslands *B* and *C*, and fruited over a considerable period, from July to October, each year. One ring has fruited every autumn over a period of 4 years. On Lakenheath Warren *M. oreades* is scarcely visible except at the time of fruiting, and if a profile through a ring is exposed no mycelium is visible. This contrasts with the growth of the fungus in lawns in Cambridge, where it may produce extensive bare patches in the turf and mycelium is often visible in the soil. Bayliss Elliott (1926) reported that *M. oreades* destroyed the grass in lawns, golf courses, and pastures, but Shantz and Piemeisel (1917) found that the fungus did not have an adverse effect on pasture in Colorado. It would appear that the fungus may react differently under different soil conditions.

Profile pits were dug through groups of sporophores of *M. oreades* growing on grasslands *B* and *C*. Soil samples were collected at successive inch depths under the sporophores, and soil plates (Warcup, 1950) were prepared in the laboratory. A difficulty was encountered in the preparation of these plates, as soil from samples taken directly below the sporophores proved very resistant to wetting. The soil had to be broken up in order to distribute the particles through the agar. At lower depths this difficulty did not arise. The soil samples were kept at laboratory temperature, and at the end of a week there was a profuse development of mycelium in the tubes from which the soil had proved difficult to wet. Table II shows the state of the soil, the growth of basidiomycete mycelium, and the number of species of microfungi recorded from soil samples collected under the sporophores of *M. oreades*.

TABLE II

Occurrence of Microfungi and the Mycelium of M. oreades *in Soil beneath its Sporophores*

	B soil			*C* soil		
Depth in inches.	Soil condition.	Mycelium.	No. of species of microfungi.	Soil condition.	Mycelium.	No. of species of microfungi.
1	Impermeable	+	5	Impermeable	+	7
2	,,	+	6	,,	+	4
3	,,	+	4	,,	+	4
4	Normal	—	18	Normal	—	13
5	,,	—	15	,,	—	15

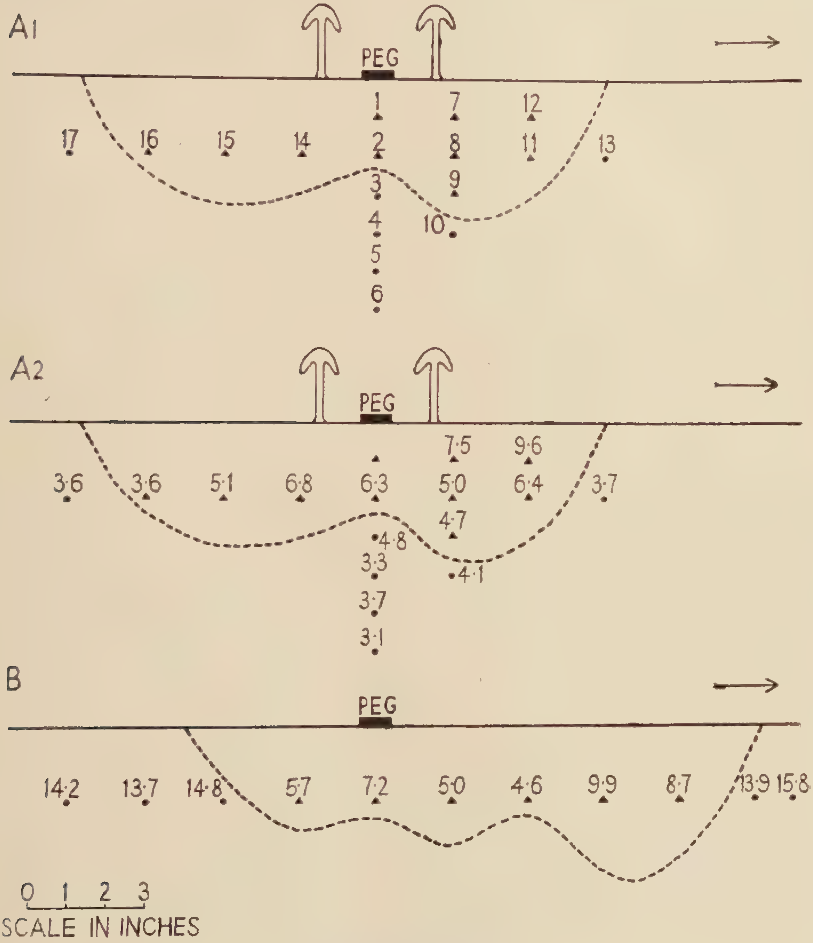
Few species (and also colonies) of microfungi were recorded in the soil just below the sporophores of *M. oreades*. This region also coincides with that in which the mycelium of the basidiomycete was found. Samples at lower depths (4 and 5 in.) showed a much greater number of microfungi, in fact the 'normal' flora for those depths in the *B* and *C* soils (Pl. XV, Fig. 1).

A more comprehensive series of samples was collected from another ring of *M. oreades*. A profile pit was dug along a radius of the ring and soil samples taken both vertically and horizontally from near the ring of sporophores. Text-fig. 2 gives a plan of the samples and shows their positions relative to the sporophores. Soil plates from all samples collected inside the mycelial zone of the agaric gave a restricted flora of microfungi while those outside the zone were normal.

The fact that soil from the mycelial zone of *M. oreades* is difficult to wet was also noted by Bayliss Elliott (1926). She considered that it was due to air trapped in the mycelium. As the soil from the mycelial zone appeared very dry, the percentage moisture contents of all the samples taken from the abovementioned ring were determined. These results are given in Text-fig. 2, A2, and show that the moisture figures for the mycelial zone are higher than those for outside. This result may be due to the very low moisture content of the whole area at the time the samples were collected. Text-fig. 2, B, shows another series of samples collected from the same ring 7 months later. In this case the mycelial zone is drier than the surrounding soil, so apparently there is impeded percolation into the mycelial zone under wetter conditions. The limits of the area occupied by the mycelium of the agaric can be plotted, particularly when the soil is wet, by the lighter colour of the soil in the mycelial zone.

During all seasons of the year further samples have been collected from soil with *M. oreades*. Eleven profiles were studied and they showed that on Lakenheath Warren the mycelium of *M. oreades* is confined to a zone near the surface of the soil from 0–0·5 to 3–4 in. deep and 6–24 in. wide. Bayliss Elliott (1926) records finding mycelium to a depth of about 12 in. Results from the soil plates of these samples showed a restricted flora of microfungi in all cases.

Some of the microfungi which were isolated from the mycelial zone of *M.*



→ Direction of growth.
--- Margin of mycelial zone.

TEXT-FIG. 2. A1, plan of soil samples collected from a ring of *M. oreades*; A2, percentage moisture figures for the soil samples from A1; B, percentage moisture figures from samples collected 7 months later.

oreades were rarely, if at all, recorded from normal soil. *Absidia*, *Fusarium*, *Gliomastix*, *Penicillium*, *Phoma*, *Mortierella*, *Stysanus*, and *Trichoderma* though of common occurrence in normal soil were not, or very rarely, found in the mycelial zone; *Penicillium nigricans* (Bainier) Thom and *Cephalosporium* were common to both; but *Gymnoascus subumbrinus* A. L. Smith and Ramsb. and *Arachniotus* were common in the mycelial zone though rarely recorded from normal soil. In a few cases *Trichoderma viride* Pers. ex Fr. was found in the mycelial zone and soon overgrew the isolation plates.

M. oreades was not often isolated from soil, and on the occasions when it did appear, it was usually as a single colony on an isolation plate. After experiments had shown that *Psalliota arvensis* grew better on soil plates if the soil had been thoroughly shaken with water (thereby wetting it) this method was tried for *M. oreades*. A better recovery was obtained, but the fungus still did not develop extensively on isolation plates. However, dead grass-roots from the mycelial zone, washed and then plated in agar, often produced cultures of *M. oreades*.

To investigate if the reduction of microfungi in the mycelial zone of *Marasmius oreades* was general or confined to the fungus growing at Lakenheath Warren, soil samples were obtained from a ring of the fungus growing on a lawn at Cambridge. As at Lakenheath, there was a great reduction in the number of species and colonies of microfungi in the mycelial zone as compared with the normal microflora of the lawn.

Psalliota arvensis

This fungus is also common on grassland *B* at Lakenheath Warren, and forms rings which are not easily distinguished except when in fruit, though the grass on a ring may be slightly darker green than usual. Bayliss Elliott (1926) records this species as attacking grass and causing bare patches. The mycelial zone of the fungus does not start at the surface of the soil but lies 1 or 2 in. deep, as has been noted by Cayley (1937). The mycelium is usually visible in the soil as white flecks and may extend to a depth of 4–8 in. Soil samples were collected from profiles through mycelial zones of *P. arvensis* and plated out as with *M. oreades*. Soil plates from the surface and the deeper samples showed (Table III) a normal microflora, but those from the mycelial zone showed the same general phenomenon as those of *M. oreades*, but with a more striking reduction in numbers of colonies of microfungi in the mycelial zone (Pl. XV, Fig. 2). The basidiomycete usually appeared on the soil plates

TABLE III

Occurrence of Microfungi and the Mycelium of P. arvensis *in Soil*

	Ring *A*		Ring *B*	
Depth in inches.	Mycelium.	No. of species of microfungi.	Mycelium.	No. of species of microfungi.
1	—	20	—	19
2	+	6	+	7
3	+	4	+	5
4	+	4	+	5
5	—	16	+	3
6	—	14	—	4
7			—	19
8			—	17

and was also isolated in profusion from roots and large humus fragments occurring in its mycelial zone. Although the fungus grew on soil plates prepared in the usual manner, it was found that more colonies were obtained if the soil was thoroughly wetted by being shaken up with sterile water before preparing the soil plates (Pl. XV, Fig. 3).

Tricholoma nudum

T. nudum was recorded on grasslands *B* and *D* at Lakenheath Warren and also occasionally in the pine forests near by. The location of its mycelial zone varied in the different soils. On the alkaline grassland *B*, the mycelial zone was 1 to 4–5 in. deep, on the acid grassland *D* the fungus occurred near the surface, 0 to 2 in. deep, and in the forest, mycelium appeared predominant in the litter zone. Soil samples through rings of *T. nudum* showed a reduced microflora in the mycelial zone (Table IV) and the basidiomycete usually grew on the soil plates (Pl. XV, Fig. 4).

T. nudum collected from fields near Wigginton, York, also showed a reduced microflora in the mycelial zone.

TABLE IV

Occurrence of Microfungi and the Mycelium of T. nudum *in Soil*

	Ring *A*		Ring *B*	
Depth in inches.	Mycelium.	No. of species of microfungi.	Mycelium.	No. of species of microfungi
1	−	14	+	5
2	+	3	+	4
3	+	5	+	4
4	+	4	+	4
5	−	15	+	5
6			−	16

Microfungi common in the Mycelial Zones of Agarics

The three fungi, *M. oreades*, *P. arvensis*, and *T. nudum*, all showed the interesting phenomenon of a reduction in the number of colonies and species of microfungi in their mycelial zone in the soil. A few species of microfungi, most of which were common to the mycelial zones of all three agarics, were found in this zone. They included several species not usually common in normal soil at that depth. Table V records the common species of fungi obtained from ten mycelial zones of each of the three agarics and from ten profile samples of normal grassland *B* soil.

Besides the ascomycetes recorded in Table V, nine other species have been recorded from the mycelial zones of agarics on Lakenheath Warren. They include *Chaetomium funicola* Cooke, *Chaetomium* spp., *Gymnoascus* (? *setosus*), *Sordaria* (? *fimicola*), and *Thielavia* sp. The occurrence and abundance of these fungi was one of the most striking features of soil plates prepared from mycelial zone soil. Also *Arachniotus* sp. and *Byssochlamys nivea* Westling were recorded from the zone of *Marasmius oreades* at Cambridge and *Chaetomium globosum* Kunze, *Gymnoascus* sp. and *Penicillium vermiculatum* Dangeard from the zone of *Tricholoma nudum* growing at Wigginton, York.

TABLE V

Common Fungi from the Mycelial Zones of Three Agarics

Culture No.	Fungus.	*M. oreades.*	*P. arvensis.*	*T. nudum.*	Normal B. soil.
B96	*Marasmius oreades*	5	0	0	0
B152	*Psalliota arvensis*	0	10	0	0
B166	*Tricholoma nudum*	0	0	8	0
B65a	*Arachniotus* sp.	5	0	6	0
B30	*Gymnoascus subumbrinus*	5	4	8	2
B78	*Cladosporium herbarum*	6	8	6	2
B13	*Penicillium* sp.	6	10	7	3
B65	*Penicillium luteum*	8	10	0	5
B16	*Thielavia* sp.	0	8	10	9
B25	*Penicillium nigricans*	6	4	10	10
B46	*Cephalosporium* sp.	9	8	6	10

The results raise the problem of whether these ascomycetes grow more frequently in the mycelial zone or whether they are only isolated more frequently. Most are slow-growing organisms which would be favoured by the low number of colonies which develop on plates prepared from mycelial zone soil. *Penicillium luteum* Zukal (Table V) has been found to be more common in grassland *B* soil than would appear from its frequency of occurrence on normal soil plates. Thus this fungus was recorded much oftener from grassland *B* soil which had been partially sterilized by steam than from untreated soil. Its ascospores are apparently resistant to heat. The resistant nature of the ascospore may also be the reason why *Penicillium luteum* survives in the mycelial zone of an agaric such as *Psalliota arvensis*, though this fungus kills most members of the microflora.

The cause of this reduction in microfungi in the mycelial zones of basidiomycetes is not known. Attempts to demonstrate an antibiotic action of *Psalliota arvensis* to the soil microflora on agar plates were without success. However, Wilkins and Harris (1944) have reported the presence of a bacteriostatic substance in sporophores and from the metabolic solution of *P. arvensis*. The test organisms were *Bacterium coli* and *Staphylococcus aureus*. A similar substance was reported for *Tricholoma nudum*, but not for *Marasmius oreades*.

Fungi from Other Localities

Two further basidiomycetes have been shown to exhibit the phenomenon of a reduced microflora in the mycelial zone.

Psalliota subfloccosa Lange:[1] in soil under beech, Haxby, York. Soil plates from the mycelial zone gave a very good growth of the agaric and few other fungi were present. *Chaetomium elatum* Kunze and Schmidt was present in the mycelial zone.

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[1] This collection was identified by E. J. H. Corner (Botany School, Cambridge). The species has not previously been recorded for the British Isles.

Psalliota xanthoderma Genev.: in litter and soil under beech, near Balsham, Camb. On soil plates the fungus grew well from leaf fragments and other larger pieces of raw humus. Few other species were present in the mycelial zone, but included *Chaetomium torulosum* Bainier.

ACKNOWLEDGEMENTS

I should like to express my thanks to Professor F. T. Brooks, under whose supervision this work was done, for his encouragement and advice; to Miss E. M. Wakefield, Dr. R. W. G. Dennis, and Mr. E. J. H. Corner, for the identification of basidiomycetes; to Dr. S. J. Hughes for the identification of *Chaetomium*; and to Mr. F. T. N. Elborn for the preparation of photographs.

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EXPLANATION OF PLATE XV

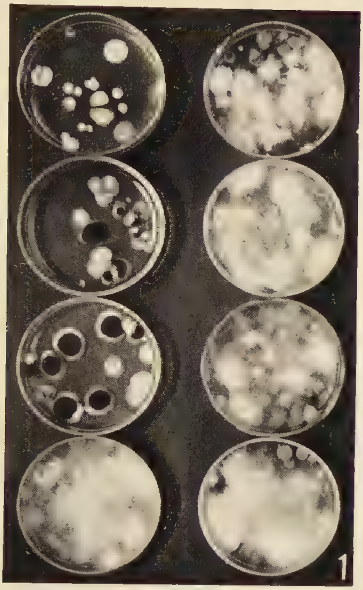
Illustrating J. H. Warcup's article on 'Studies on the Growth of Basidiomycetes in Soil.'

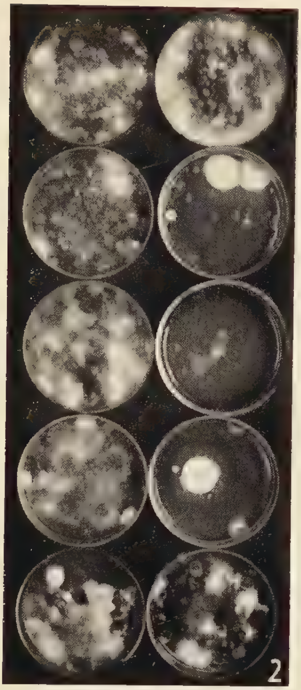
Fig. 1. Soil plates from soil samples collected at 1-in. intervals, after 3 weeks, (left) under *Marasmius oreades*, (right) normal soil.

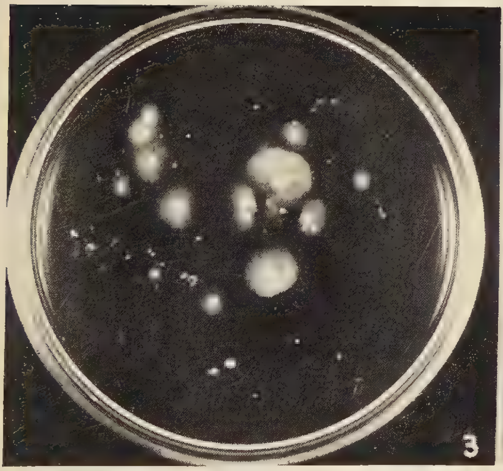
Fig. 2. Soil plates from soil samples collected at 1-in. intervals, after 3 weeks, (left) normal soil, (right) under *Psalliota arvensis*.

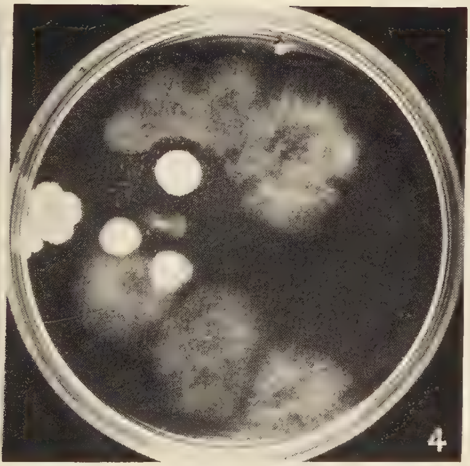
Fig. 3. Colonies of *Psalliota arvensis* on a soil plate, after 3 weeks.

Fig. 4. Tenuous colonies of *Tricholoma nudum* on a soil plate, after 3 weeks.









J. H. WARCUP

Experimental Studies of the Relation between Carbon Assimilation and Stomatal Movement

II. The Use of the Resistance Porometer in estimating Stomatal Aperture and Diffusive Resistance

Part 2. SOME SOURCES OF ERROR IN THE USE OF THE RESISTANCE POROMETER AND SOME MODIFICATIONS OF ITS DESIGN

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With three Figures in the Text

ABSTRACT

Two purely physical sources of error in the 'resistance porometer' as ordinarily used are considered to be of appreciable importance, viz. that due to the flow through the leaf being augmented with water vapour which is removed again by a drying agent before the air passes through the standard capillary, and that due to the leaf temperature differing from the capillary temperature. In a bad case the total error might amount to 10 or 15 per cent. of the 'leaf resistance'.

Modifications to deal with these sources of error and with the more important errors due to the responses of the enclosed stomata to variations in CO_2 supply are described.

INTRODUCTION

IN an earlier paper (Heath, 1941) one of us investigated the problem of determining in absolute terms, by means of the 'resistance porometer' (Gregory and Pearse, 1934), the resistance of an average stoma to viscous air-flow. The mathematical theory of porometers elaborated for this purpose by Penman (1942) was used to eliminate the effects of the irrelevant resistances due to the intercellular space system and the stomata outside the cup on both surfaces of the *Pelargonium* leaf. The importance of obtaining such absolute determinations of stomatal resistance to *viscous flow* lies in this being a necessary prerequisite to the calibration of the resistance porometer in terms of diffusion, to yield similar estimates of stomatal resistance to *diffusive flow*. These would clearly be of the greatest value in the experimental investigation of problems of stomatal control of carbon dioxide assimilation or transpiration. Such calibration should be possible if alternate readings for the same

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porometer cup are made with the resistance porometer and the 'diffusion porometer' (Gregory and Armstrong, 1936), and a considerable body of data (Heath, unpublished) was collected in this way for *Pelargonium*.

For the valid use of Penman's theory certain conditions must be satisfied, one being that the mean stomatal apertures, or more precisely their resistances, are uniform over the leaf. More recent work (Heath and Williams, 1948; Williams, 1949; Heath, 1950) has shown that with a conventional porometer cup permanently attached to the leaf this condition is by no means fulfilled, the enclosed stomata within the cup opening in the light to a very much greater extent than those elsewhere on the leaf. In the preliminary note announcing this finding (Heath and Williams, loc. cit.) are mentioned certain modifications of the design of the resistance porometer, intended to ensure that the conditions within and above the porometer cup are as similar as possible to those for the rest of the leaf; these modifications are described in detail below together with a somewhat simpler type of detachable cup than that used by Spencer in this Institute before the war and described by Gregory et al. (1950). With the porometer in its modified form the striking differences in stomatal aperture within and outside the cup are no longer found.

In addition to the source of error mentioned above, which was biological in origin and resulted from the sensitivity of the stomata to reduction of the CO_2 content of the air (Heath, 1948, 1950; Heath and Milthorpe, 1950), it seemed that certain purely physical sources of error might be important, especially where the temperature of the leaf was considerably higher than that of the standard capillary resistance of the porometer. Some such ideas appear to have occurred also to the writers of a new text-book (Curtis and Clark, 1950, p. 226). A theoretical investigation of the probable magnitude of such errors given below suggests, however, that they are unlikely to exceed 15 per cent. of the measured leaf resistance even in bad cases, and with reasonable care should not exceed a few per cent. Experiments to test the magnitude of the expected errors confirmed that they were small. It seems, therefore, that the resistance porometer, as modified, may be safely used to estimate stomatal resistance to viscous flow. The calibration in terms of diffusion requires further experimental work using a detachable cup, for the data mentioned earlier were collected using permanently attached cups, and the apertures of the enclosed stomata were almost certainly different from those on the rest of the leaf.

THEORETICAL

In deriving the formula used with the resistance porometer for calculating the leaf resistance use is made of the Ohm's Law relation applied to gaseous flow (cf. Heath, 1941, p. 462). The equation for the flow of gases, where the flow is streamlined and the mean free path of the molecules small compared with the dimensions of the tube, may be written (Loeb, 1934)

$$G = \frac{\pi r^4}{8\eta L}(p_1 - p_2)p\frac{M}{RT}, \qquad (1)$$

where G = mass of gas flowing in unit time, r = radius of tube, L = length of tube, η = viscosity of gas, p_1, p_2 = pressures at ends of tube, $p = \frac{1}{2}(p_1+p_2)$ = mean pressure of gas, M = molecular weight of gas, T = absolute temperature, R = gas constant per gram-molecule.

Transposing (1) into the familiar Ohm's Law form ($E = IR$) we can write

$$p_1-p_2 = \frac{G}{M}\left(\frac{RT}{p}\frac{8\eta}{\pi}\frac{L}{r^4}\right), \tag{2}$$

where the 'current' G/M is for convenience expressed in terms of gram molecules. In the previous treatment (Heath, 1941, p. 464) 'resistance' was defined as the purely dimensional constant L/r^4 and it was assumed that the viscosity η of the gas, the temperature T, and the mass of gas flowing G, were all constant throughout the system. For reasons which will shortly be apparent it is here proposed to treat η, T, and G as variables and to define 'resistance' as $(p_1-p_2)\div G/M$.

The viscosity of a gas varies as $T^{\frac{1}{2}}/(1+K/T)$ (Loeb, 1934), where K is a constant (Sutherland's constant). Thus the resistance Z of the tube as just defined is given by

$$Z = \frac{RT}{p}\left\{\sqrt{\frac{T}{T_0}}\,\frac{1+(K/T_0)}{1+(K/T)}\,\eta_0\right\}\frac{8}{\pi}\frac{L}{r^4}, \tag{3}$$

where η_0 is the viscosity at $T_0 = 0°$ C.

Now it will be recalled that in the resistance porometer a current of air flows through a fine glass capillary after passing through the leaf. In effect, the pressure drops $\Delta_1 p$ and $\Delta_2 p$ across the capillary and leaf respectively are observed. If Z_1 and Z_2 are the corresponding 'resistances' as defined above, and G/M is the rate of mass-flow of the air, then we have

$$\Delta_1 p = \frac{G}{M}Z_1, \tag{4a}$$

$$\Delta_2 p = \frac{G}{M}Z_2, \tag{4b}$$

and Z_2 can be found in terms of Z_1, $\Delta_1 p$, and $\Delta_2 p$:

$$Z_2 = Z_1\frac{\Delta_2 p}{\Delta_1 p}. \tag{5}$$

This is equivalent to equation (ii) of the former paper (Heath, 1941, p. 462).

It will be seen that a number of new assumptions are involved in the derivation and use of this formula, which amount to the following:

(1) That the flow G/M is the same in both leaf and capillary.
(2) That the resistance of the capillary constitutes a fixed standard.

These two points will now be examined in turn.

Difference in mass-flow in leaf and capillary

The air-stream through the leaf and capillary may be in either direction (see next section), although the arrangement of the apparatus has hitherto been as shown diagrammatically in Fig. 1.

Now it will be noticed that whereas with this apparatus the gas-stream through the capillary is quite dry (necessary to prevent condensation), that through the leaf is bound to be moist over at least a part of its journey. Even in the case where the air flows *into* the leaf from the porometer and so enters the stomata within the cup in a dry state, it rapidly becomes moist within the leaf and this fact constitutes an increase in the current G/M. The worst case will clearly occur when the air throughout its course in the leaf is fully saturated, as this will give the greatest discrepancy between the gas currents in the leaf and in the capillary. Suppose that the air-flow in the capillary is G/M, the atmospheric pressure being P. Then if the 'mean' vapour pressure in the current in the intercellular spaces is p_v, the air will here be approximately at the pressure $P-p_v$ but its mass-flow will still be G/M. Accompanying this, however, will be a flow of vapour equal to $\{p_v/(P-p_v)\}G/M$, since in a given space the numbers of gram-molecules of different gases are directly proportional to their partial pressures. Hence the flow in the leaf will be

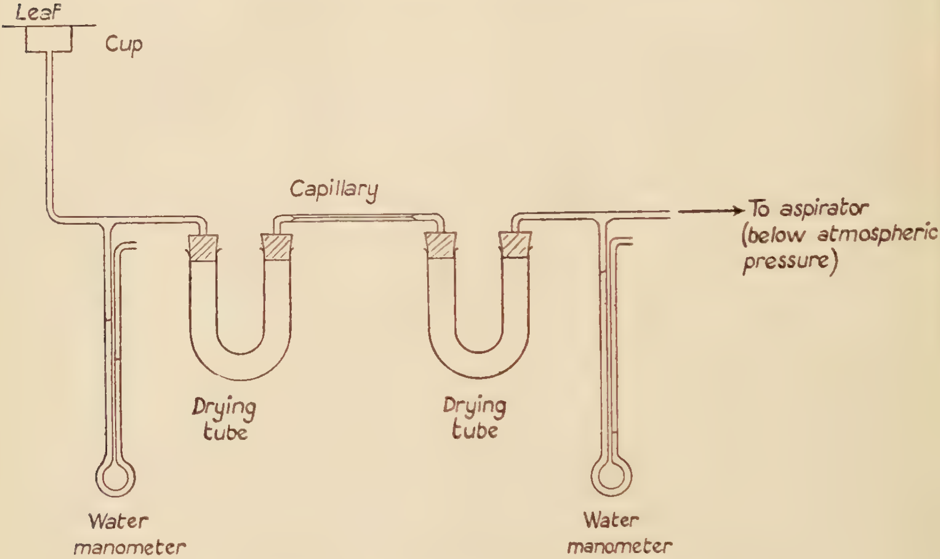

FIG. 1. Diagram of the resistance porometer in its usual form.

$$\frac{G}{M}+\left(\frac{p_v}{P-p_v}\right)\frac{G}{M}=\left(\frac{P}{P-p_v}\right)\frac{G}{M},$$

and (neglecting the very slight change in Z_2 due to alteration of the composition of the gas-stream), equations (4 *a*) and (4 *b*) become

$$\Delta_1 p = \frac{G}{M} Z_1,$$

$$\Delta_2 p = \frac{G}{M}\left(\frac{P}{P-p_v}\right) Z_2,$$

from which

$$Z_2 = \left(\frac{P-p_v}{P}\right)\frac{\Delta_2 p}{\Delta_1 p} Z_1 \tag{6}$$

instead of the commonly calculated value

$$Z_2' = \frac{\Delta_2 p}{\Delta_1 p} Z_1. \tag{5}$$

To see the order of magnitude of this effect suppose that the air during its course through the leaf has a 'mean' saturation of 80 per cent. at a temperature of 25° C., the atmospheric pressure being 760 mm.

Then $p_v = 0{\cdot}80 \times 23{\cdot}8 = 19$ mm. and

$$\frac{P}{P-p_v} = \frac{760}{741} = 1{\cdot}026.$$

Thus the calculated resistance Z_2' will be about 2½ per cent. too high. Had the temperature been 30° C. the error would have been 3½ per cent., and if the air had been quite saturated at 30° C., about 4½ per cent. In general it can be said that this source of error increases rapidly with temperature and is nearly proportional to the increase in relative humidity of the air-stream in the leaf. It may be expected to be reduced when the air-flow is from the porometer to the leaf; the air will then traverse in a relatively dry state both the capillary tube and the stomata within the cup, which last in *Pelargonium* normally constitute the most resistive part of its course through the leaf and especially so at small stomatal apertures.[1] Apart from this last effect, however, for a given value of the 'mean' vapour pressure in the leaf, the percentage error of the calculated resistance will be the same at all stomatal apertures.

Variations in the standard capillary resistance

On examining equation (3) it will at once be seen that the 'resistance' of a capillary tube varies with the following factors:

1. *Temperature.* The resistance increases with temperature due to the expansion of the gas. There is a second effect due to the temperature coefficient of viscosity. This is practically the same for all the common gases, although 'Sutherland's constant' K varies somewhat from gas to gas. Apart

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[1] Cf. Heath, 1941, Table I, p. 473. The lowest resistances in the table do not occur except with permanently attached and unswept porometer cups. The value of m used, although now open to some doubt, is apparently of the right order.

from changes in composition of the air-stream, however, the effect of temperature is the same on all elements of the path, and so cancels out unless temperature differences appear.

2. *Pressure.* The resistance varies inversely with the mean pressure p. If the flow be expressed in terms of volume of gas flowing at this pressure, the resistance becomes independent of p, but the flow is not then constant in different parts of the path and it is more convenient to express it in terms of gram-molecules.

3. *Nature of the gas.* This, of course, affects the viscosity.

These points will be dealt with in turn, taking 2 and 3 first.

Variation of pressure is usually quite unimportant and may be disregarded. The total pressure difference involved is usually only a few centimetres of water, and expressed as a percentage of atmospheric pressure the difference between the centre of the capillary and the open air would commonly be of the order of only $\frac{1}{4}$ per cent. or so.

So far as the nature of the gas is concerned this, too, is unimportant. The gas passing through the capillary is usually dry air. On the other hand, the gas passing through the leaf is moist air with a viscosity which is probably slightly lower than that of dry air (but see 'International Critical Tables', 1927, v. 6). Spurious indications of change in stomatal resistance will thus be liable to appear if the 'mean' absolute moisture content of the air within the leaf changes during an experiment. This can most easily result from a rise in temperature of the leaf which will increase the absolute humidity of the air in the leaf and so lower (probably) its viscosity. The effect, however, will be small and in any case difficult to evaluate. It should be distinguished from another effect discussed below—that of the variation of viscosity with temperature as opposed to its variation with composition of the air-stream. It may be mentioned that no error will be involved in substituting another gas (say pure nitrogen or hydrogen) for air: spurious changes in calculated resistance only arise when the composition of the gas varies *differentially* in the leaf and capillary. In practice this is only likely to occur through changes in the moisture content of the stream traversing the leaf.

The effect of temperature is rather complex. Indirectly it causes variations in the mass-flow and in the viscosity as discussed above, both of these effects depending on changes in the absolute moisture content of the air-stream. However, it also has two direct effects: rise in temperature changes the viscosity of the gas, and it also causes an increase in volume. For a given mass-flow the volume flow will therefore be greater and the pressure drop correspondingly increased. Therefore the resistance as defined by the relation $Z =$ pressure drop/mass-flow will be increased. Both of the above direct effects of temperature are included in the equation (3), which indicates that resistance increases with temperature according to the function $T^{\frac{3}{2}}/(1+K/T)$. To see what this means consider a typical case. Let the resistance of a tube to air at 20° C. be taken as unity. Then if K be assumed equal to 120, the resistances at other temperatures work out as follows:

Temperature, °C.	Resistance
0	0·881
10	0·940
20	1·000
30	1·062
40	1·125

Since all 'resistors' will have the same 'temperature coefficient' of resistance, the value of the leaf resistance in terms of the standard capillary will be independent of the temperature of the experiment, *provided both leaf and capillary are at the same temperature.* Suppose, however, that this is not the case, but that while the capillary is at 20° C. the leaf is at 30° C. Then clearly the calculated value of the leaf resistance will be about 6 per cent. too high, and, moreover, fluctuations in leaf temperature caused by variations in light intensity and transpiration will cause spurious fluctuations in the porometer reading.

Conclusions

The above results may be summarized as follows.

1. If the air in the capillary is dry (as is usually the case) the leaf resistance as calculated ordinarily will be too high owing to the gas-flow through the leaf being augmented with water vapour. The error will probably be less when the air is 'pushed' first through the capillary and then through the leaf, rather than 'pulled' in the reverse direction, owing to the fact that relatively dry air will then be entering through the stomata within the cup. In a typical case the error might be about 3 per cent.

2. If the leaf is allowed to assume a different temperature from the capillary, its calculated resistance will be higher or lower than the true value, depending on whether its temperature is higher or lower than that of the standard. If it is 10° C. higher (as might easily be the case under high illumination), the resistance found will be about 6 per cent. above its true value. Variations in temperature of the leaf or capillary will cause spurious variations in the porometer reading.

3. The resultant error due to both the above effects (for which a product relationship holds)[1] might reach as high as 10 or even 15 per cent. in a bad case, but in actual practice with reasonable care it should not be more than a few per cent.

APPARATUS

As is indicated in the preceding section, in order to reduce errors due to the passage of dry air throughout the capillary and moist air through the leaf, a 'push' porometer, as it may be termed, which forces dry air into the leaf from the porometer cup with a pressure above atmospheric, is preferable to the usual 'pull' porometer in which moist air is drawn into the cup from the leaf with a reduced pressure. A further advantage of the 'push' porometer, however, is that the drying-tubes on either side of the standard capillary resistance can be dispensed with and *moist* air used as long as the relative humidity is well below saturation so that no condensation occurs; thus the difference between the mass of gas passing through the capillary and the leaf can be very greatly reduced. With the usual 'pull' porometer air emerging from the leaf is usually saturated at a temperature higher than that of the capillary and a drying-tube is therefore essential.

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[1] e.g. leaf at 30° C. with saturated air, capillary at 20° C. with dry air:
total error = $1{\cdot}045 \times 1{\cdot}062 = 1{\cdot}110$, or 11 per cent.

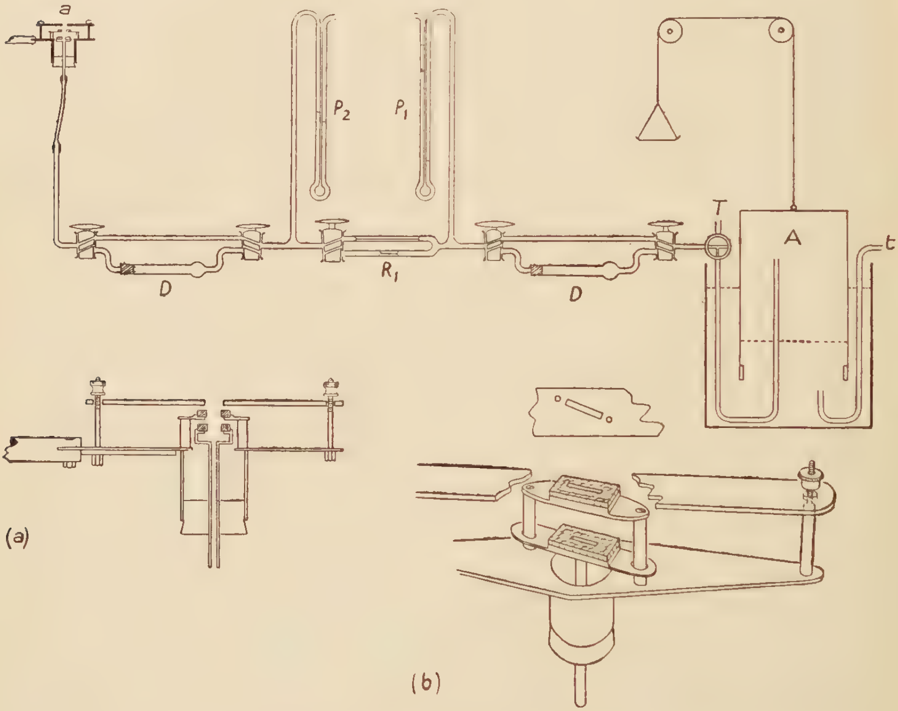

FIG. 2. The modified resistance porometer. *a*, Circular detachable cup; *b*, rectangular detachable cup for use with wheat leaves.

The modified resistance porometer as now used is shown diagrammatically in Fig. 2. A simple 'gasometer' type of aspirator A is made from a suitable tin canister weighted at the open end with 'compo' tubing and suspended over ball-bearing pulleys in a bath of medicinal paraffin. It is normally used to provide a pressure of 5–10 cm. of paraffin above atmospheric, but by placing weights in the pan it can be adjusted to give a reduced pressure for a 'pull' porometer—it is then usually necessary to remove some of the paraffin from the bath in order to prevent it from entering the air tube. The pressure provided by the aspirator is nearly constant and changes solely owing to the change in that volume of the tin which is totally immersed; since the walls can be very thin this change is small and pressure changes can in any case be read from manometer p_1. This type of aspirator has the great advantage over the usual 'constant pressure' aspirator that there is no bubbling of the air through liquid and hence the menisci in the manometers are absolutely steady and can be read instantaneously. For use with the 'push' porometer the aspirator is filled through tap T, either with ordinary laboratory air or air at any other desired humidity and CO_2 content. Alternatively the reservoir can be refilled by forcing the desired air-supply in through tube t under slight pressure, as with a scent-spray bulb. The air passes from the aspirator to the porometer, which resembles that previously described (Heath, 1941), except that light medicinal paraffin (s.g. 0·83) is used instead of water in the manometers p_1 and p_2 and that the drying-tubes DD are normally by-passed out of the path of the air-stream. The manometer readings are of opposite sign to those obtained with the 'pull' porometer, since all the pressure gradients are reversed, but they are used in the usual way for calculation of the leaf resistance (see Fig. 3).

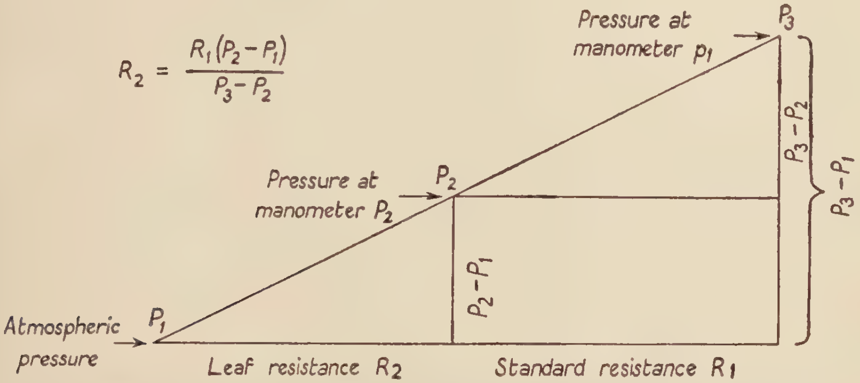
$R_2 = \frac{R_1(P_2 - P_1)}{P_3 - P_2}$
Pressure at manometer p_1
P_3
$P_3 - P_2$
$P_3 - P_1$
Pressure at manometer P_2
P_2
$P_2 - P_1$
Atmospheric pressure
P_1
Leaf resistance R_2
Standard resistance R_1

FIG. 3. Diagram illustrating the pressure relations of the modified resistance porometer (cf. Heath, 1941, Fig. 5, p. 464).

Undoubtedly the most important modifications of the porometer lie in the use of a cup detached from the leaf between readings, thus exposing the area within the cup to the same conditions as the rest of the leaf, and also in the case of amphistomatous leaves in the exposure of the upper leaf surface immediately above the cup. For hairy leaves such as *Pelargonium*, and even for wheat, it is necessary to use a special design of detachable cup which leaves the gelatin washer in position on the leaf when the main part of the cup is removed. Such a cup was used in this Institute before the war by Spencer for *Pelargonium*, and recently by Milthorpe for wheat (Gregory et al., 1950). Since these detachable cups were used inside special leaf chambers they were necessarily of somewhat elaborate design, and two simpler cups which have proved satisfactory, a circular cup for *Pelargonium* and a rectangular one for wheat, are shown in Fig. 2. Attention must be called to the hole cut in the Perspex plate above the cup area in each case; this exposure of the upper surface is of great importance even with *Pelargonium* (Heath, 1950), while in the case of wheat, where nearly all the air must pass straight through from bottom to top owing to the large number of upper stomata (Heath and Milthorpe, 1950), it is most essential. In some cases a small thermocouple for taking leaf temperatures has been used. This enables the difference in temperature between leaf and capillary to be estimated and a correction applied to the measured leaf resistance if desired. The thermocouple is soldered to a small piece of silver foil which is pressed against the leaf surface within the cup and the leads are sealed with wax through a hole in a thin Perspex washer fixed to the brass plate which holds the gelatin washer against the leaf. This brass plate and gelatin washer should be as thin as possible, say 3–4 mm. in all, in order to expose the enclosed leaf surface adequately. The detachable part of the cup, which carries a second gelatin washer to make an airtight seal with the brass plate or Perspex washer, can be raised or lowered by sliding the stem in a rubber bung. The stem should be an extremely tight fit in the hole in the bung and should be lubricated with a touch of 'Silicone' tap grease. Both gelatin washers are coated with the usual 'Vaseline'-beeswax luting wax to give airtight seals.

DISCUSSION

The modifications in the design of the resistance porometer described above were originally made to eliminate the errors due to the responses of the stomata within the cup to changes in the CO_2 content of the enclosed air. The relevance of such errors to investigations with the porometer has been discussed in detail elsewhere (Heath, 1950); they are probably of much greater importance than the relatively small physical errors discussed in the 'Theory' section above. For eliminating errors due to the CO_2 effect the type of detachable cup first introduced by Gregory and Spencer (Gregory et al., 1950) and the exposure of the leaf surface above the cup seem to offer the most satisfactory method. It was indicated by direct microscopical observation (Heath, 1950) that a gelatin washer fitted against the leaf had no differential effects upon the stomata within and outside the encircled area as long as the air within the washer was not totally enclosed. Observations by Lloyd's (1908) method of stomatal apertures within and outside detachable cups fitted to *Pelargonium* leaves have also failed to show any consistent differences. A cup swept with air such as surrounds the rest of the leaf, as used previously (Heath, 1950), also results in stomatal apertures of similar magnitude inside and outside the cup, but in theory at least there is always a risk of sweeping too fast or too slowly and the detachable cup is preferable. Unfortunately the use of either a swept cup or one detached between readings, which is essential in any quantitative work with the porometer, renders continuous readings impossible.

A 'push' type of porometer also at first seemed desirable owing to the CO_2 effect on the stomata, for it enables air of the same CO_2 content as that surrounding the rest of the leaf to be supplied to the cup, while with the 'pull' porometer the CO_2 content of the air entering the cup is modified by passage through the leaf; this last effect, however, becomes of minor importance with a cup detached (or swept) between readings. The 'push' porometer, however, offers the real advantage that moist (but not saturated) air can be passed through the standard capillary; this, coupled with the fact that such air will enter the stomata within the cup before becoming saturated, should render the errors due to difference of mass-flow through the capillary and leaf of negligible proportions.

The use of medicinal paraffin in the aspirator and manometers not only makes it possible to use air of any desired humidity but reduces the risk, present with many organic liquids, of affecting the stomata by volatile substances. The steadiness of the pressure produced by the aspirator and the absence of 'sticking' of the manometer columns, so prevalent with water manometers, both contribute to more accurate readings.

The temperature effect is likely to be more important than the humidity effect, and it is clearly desirable to maintain the leaf and capillary at nearly the same temperature. It is thus particularly unfortunate that Wilson (1948) should have gone to the trouble of maintaining the standard capillary of his resistance porometer at constant temperature when investigating the effects of temperature on the stomata. Where large changes of leaf temperature are expected, as with a wide range of light intensities or under conditions favouring large changes in transpiration, it is desirable to use a fine thermocouple within the cup to estimate leaf temperature and if necessary to apply a correction.

It should perhaps be emphasized that the humidity and temperature effects have been discussed as affecting the *total* leaf resistance measured (R_2 of Heath, 1941). The errors due to the humidity effect are likely to vary in different parts of the leaf as the humidity of the air increases; it is therefore particularly desirable to eliminate as much as possible of this source of error by using moist air in a 'push' porometer if the data are to be subjected to the type of analysis developed by Penman. In the case of the more important temperature effect, since the flow is normally very slow and the stomata scattered, it seems likely that the air will take up the leaf temperature (to a near approximation) before it actually enters the leaf; thus the flow into, through, and out of the leaf will be more or less isothermal and the analysis of the total resistance into its components will not be invalidated.

SUMMARY

Several purely physical sources of error in the 'resistance porometer' as ordinarily used are considered theoretically and two of them are thought to be of more than negligible importance. These are:

1. That if dry air is drawn through the standard capillary, the leaf resistance will tend to be over-estimated owing to flow through the leaf being augmented

with water vapour. This error will increase with temperature, and in a typical case might amount to some 3 per cent. at 25° C.

2. That if the leaf has a temperature above or below that of the capillary its resistance will be over- or under-estimated. If the leaf temperature exceeds the capillary temperature by 10° C., the resistance will be over-estimated by about 6 per cent. Variations in leaf temperature (or capillary temperature) will cause spurious variations in apparent leaf resistance.

The total error due to both these effects might amount to 10 per cent., or even 15 per cent. in a bad case.

Modifications in the design of the resistance porometer, to deal with these sources of error and with the more important errors due to the responses of enclosed stomata to variations in CO_2 supply, are described. These consist in:

a. The reversal of the direction of air-flow through the porometer and the use of a 'gasometer' type aspirator to supply *moist* air through the capillary, medicinal paraffin being used both in the aspirator and the porometer manometers. This not only should eliminate most of the water-vapour error but appreciably increases the ease and accuracy of reading the manometers.

b. The use of a fine thermocouple in the cup to estimate differences between leaf temperature and capillary temperature. A correction may thus be made if necessary.

c. The use of a simple type of detachable cup to eliminate the CO_2 error by exposure between readings of the leaf surface within and above the cup.

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Studies in the Genus *Elaphoglossum* Schott

II. The Root and Bud Traces

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With thirteen Figures in the Text

SUMMARY

Further investigations upon some Jamaican species of *Elaphoglossum* have shown that, parallel to a series of stelar forms demonstrated earlier, there is another series showing a progressive reduction of the lateral bud trace. The anatomical differences between the root and bud traces are indicated and the development of the stele in a young outgrown bud is described.

(i) THE ROOT TRACE

ALL the roots in *Elaphoglossum* are adventitious. In *E. latifolium* and in other species with a creeping rhizome the primary adventitious roots reach up to 1 mm. in diameter. In other species the roots tend to be thinner. In all species the roots branch freely into rootlets of the third and fourth orders; they are all brown in colour and tough and wiry. The root-hairs are often very persistent and may even be found on the old adventitious roots close to the rhizome.

Anatomy. The general structure of the root of *Elaphoglossum* does not differ from that commonly found in the Leptosporangiate ferns (Ogura, 1938), although there are a few features which have not been previously described. All the roots show a diarch xylem strand with exarch protoxylem and a very conspicuous inner fibrous cortex.

The ultimate rootlets show the same general structure, but are somewhat simpler. In some, such as that of which a transverse section is shown in Fig. 1, there is no lignification of the xylem, although the general outline of the radial plate is still evident. The endodermis is ill developed and may have disintegrated. The inner fibrous cortex is much reduced and consists at the most of two layers of cells. Opposite the poles of the diarch plate, where the protoxylem would normally be differentiated, the fibrous zone is broken by thin-walled cells, reminiscent of the 'passage cells' of Angiosperm roots.

The anatomy of rootlets associated with the buds is quite similar to that of other roots.

Branching. Lateral branches of the root trace arise in the region of one of the exarch protoxylem groups of the parent trace. For this reason groups of

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lateral roots frequently appear in a distichous arrangement. The endodermis of the branch and the fibrous inner cortex are continuous with that of the parent root. As has been reported for other Leptosporangiate ferns (Ogura, 1938), the plane of the diarch xylem plate of the branch is perpendicular to that of the parent axis.

Infection. The outer, unthickened, layers of the cortex appear always to be infected with fungal hyphae. It has not been possible to determine from the preserved material whether this is a stage in the necrosis of the outer tissues or whether there is an initial phase of balanced parasitism and symbiosis.

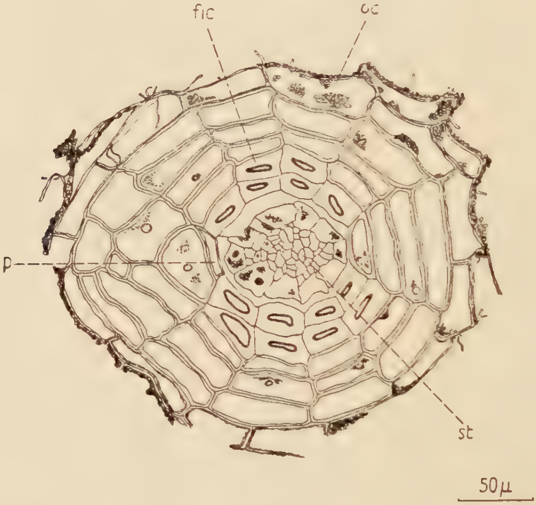

FIG. 1. *Elaphoglossum latifolium.* Diagram of a transverse section of a tertiary rootlet. *oc*, outer cortex, partially necrotic and containing fungal hyphae; *fic*, fibrous inner cortex; *p*, thin-walled cell opposite region where protoxylem would normally be developed; *st*, undifferentiated tissue of stelar region.

The course of the root trace within the cortex of the rhizome. In *E. latifolium* the root trace is found to run obliquely forward from its insertion on the stele until its emergence from the rhizome. This appears to be the general rule in all the Jamaican species, although variations are not uncommon. In *E. chartaceum* root traces have been observed running obliquely backwards from their insertions to the point of emergence. In other species traces have been found which run obliquely forwards and then backwards. In *E. villosum* the root trace normally ascends from its insertion, bends outwards, and emerges horizontally.

The root trace has not been seen to branch while it is within the cortex of the rhizome, although branches may arise very soon after its emergence.

The anatomy of the root trace within the rhizome. The root trace departs from the smaller outer tracheids of the ventral meristele of the rhizome. In its early stages the root trace bears little resemblance to the trace emerging from the rhizome. This change in the anatomy of the root trace as it passes outwards through the cortex of the parent rhizome or stem has been noted in other ferns, both living (Lachmann, 1889) and from the Tertiary (Kidston and Gwynne-Vaughan, 1911).

A transverse section of a root trace in *E. latifolium* near its insertion on the stele is shown in Fig. 2. The trace is roundly elliptical with its long axis tangential to the rhizome. Protoxylem elements occur around the periphery of the xylem mass, which includes a number of undifferentiated parenchymatous cells. The phloem, separated from the xylem by a parenchymatous zone, extends more or less continuously around the xylem. The whole stele is bounded by a conspicuous endodermis and is embedded in the cortex of the rhizome. The root trace was found to have this structure in this region in other species.

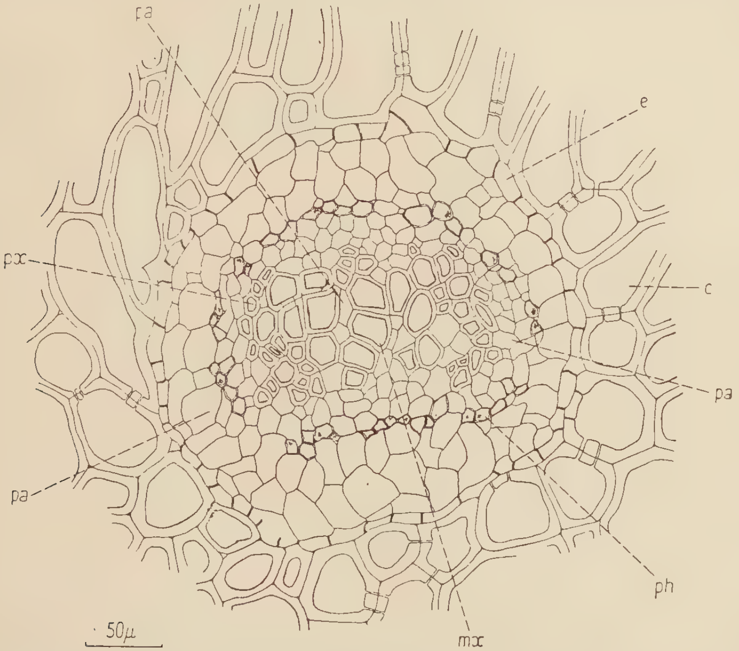

Fig. 2. *Elaphoglossum latifolium.* Diagram of a transverse section of a root trace in the cortex of the rhizome near its insertion on the stele. *c*, cortex of rhizome; *e*, endodermis; *pa*, parenchyma; *ph*, phloem; *px*, protoxylem; *mx*, metaxylem.

As the trace passes outwards, the xylem comes to assume the form of a radial plate with two protoxylem groups. At the same time the trace acquires an investment of fibrous cells, continuous with the fibrous inner cortex of the root. This zone of fibrous cells is a striking feature of the root trace just before its emergence.

A peculiar feature was observed in *E. tectum.* At a short distance behind the apex of the rhizome a freely branching and, apparently, quite normal root was sectioned close to its emergence. The vascular tissue of the root was found to be undifferentiated, shrunken, and lying loosely within the central cavity, although the fibrous inner cortex was developed quite normally. Within the cortex of the rhizome the trace passing to this root was only partially differentiated (Fig. 3). Protoxylem was present, but the central region was occupied by thin-walled parenchymatous cells. The investment of fibrous cells was, however, present in undiminished amount.

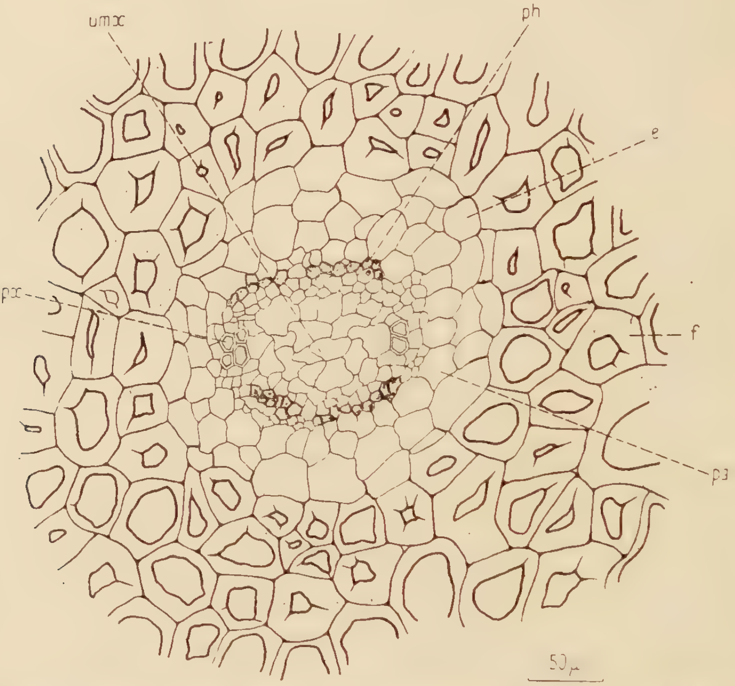

Fig. 3. *Elaphoglossum tectum.* Diagram of transverse section of a root trace in the cortex of the rhizome just before its emergence. *f*, zone of fibrous cells continuous with the fibrous inner cortex of the root; *e*, endodermis; *pa*, parenchyma; *ph*, phloem; *px*, protoxylem; *umx*, undifferentiated tissue in the region normally occupied by metaxylem.

Fibrous cells appear always to accompany the root trace in the outer cortex of the rhizome. This feature has been of importance in determining the morphological nature of rudimentary traces in association with buds.

(ii) The Bud Trace

In a previous paper (Bell, 1950) the presence of dormant buds was recorded in a number of species of *Elaphoglossum* which showed a wide range of stelar structure. These buds occur on the abaxial side of the petioles and may be conspicuous, as in *E. latifolium* (Bell, 1950, Fig. 2), or very inconspicuous, as in *E. villosum* (Fig. 4). It has been found that the development of the bud trace varies in relation to the stelar structure described in the earlier paper.

General form of the bud trace. The bud trace usually separates as a loop of a meristele of the parent rhizome and is thus frequently U-shaped at its origin. In its outward course the arms of the U close, giving rise to a protostelic or solenostelic trace. Occasionally, where it is ill developed, the bud trace may be solid from its origin. In those species with creeping rhizomes and prominent dormant buds it is usual to find a solenostelic bud trace. In other species it is usually protostelic. In *E. villosum* the bud trace tends to be slightly U-shaped at first, but rapidly contracts to form a tenuous simple strand.

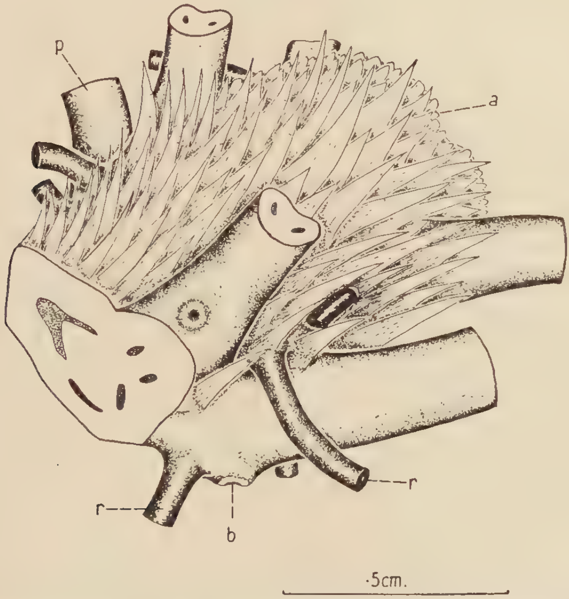

FIG. 4. *Elaphoglossum villosum.* Apical portion of rhizome showing dormant buds. *a*, apex; *p*, petiole; *r*, root; *b*, bud.

Anatomy of the bud trace. A drawing of a transverse section of a bud trace in *E. chartaceum* just before its entering the bud is shown in Fig. 5. The trace was solenostelic. A more or less continuous zone of xylem was present with the protoxylem elements principally periphical. Parenchymatous tissue surrounded the xylem and separated it on both sides from a band of phloem. The vascular tissue was again surrounded by parenchyma and the whole trace bounded by a well-marked endodermis. Outside the endodermis was the normal cortical tissue of the rhizome; there was no trace of the fibrous cells characteristic of the root trace. In this section a group of tracheids was seen separating from the stele; these formed a trace running out to one of the small roots just behind the bud.

In *E. muscosum* there was considerably less development of the bud trace.

A transverse section at the base of a well-developed bud is shown in Fig. 6. Here the bud trace was much smaller in section than that of *E. chartaceum.* The xylem was less continuous and the phloem little developed. A group of tracheids was seen passing off as a root trace, and it was interesting to note that, although the bud trace had become very reduced, the root trace still remained clearly distinguishable. In a section cut a little nearer the apex of the bud than the one figured here the root trace, although showing the differentiation of only a few tracheids, was nevertheless surrounded by the characteristic fibrous cells.

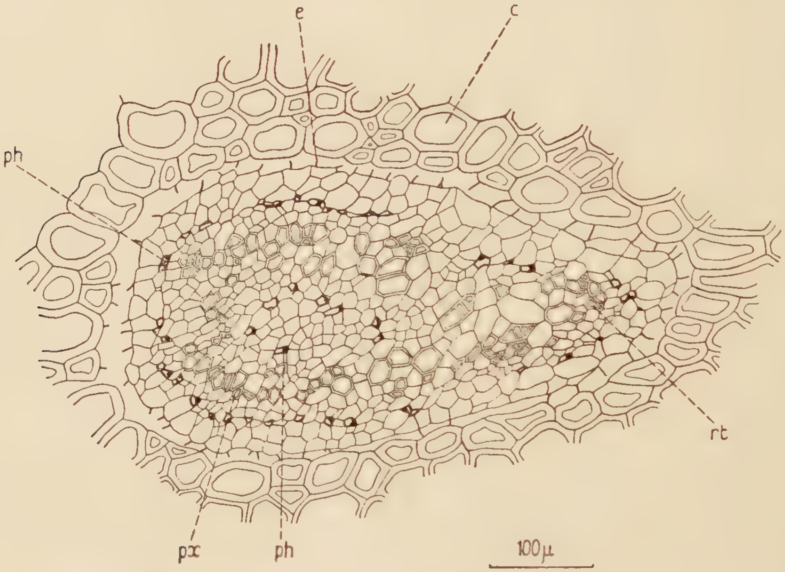

FIG. 5. *Elaphoglossum chartaceum.* Drawing of a transverse section of the bud trace just entering the bud. *c*, cortex of rhizome; *e*, endodermis; *rt*, root trace; *ph*, phloem; *px*, protoxylem.

E. hirtum resembled *E. muscosum* in the development and anatomy of the bud trace.

In *E. pallidum* there was even further reduction of the trace. The petiolar buds in this species were evident, though small. A drawing of a surface section of the abaxial side of a petiole, cutting the bud and an associated root just beneath it in a transverse plane, is shown in Fig. 7. The root associated with the bud was abortive and showed only a stump-like development externally, but the fibrous cortex was present and unambiguous, although there was little differentiation of stelar tissue within the trace. The bud trace was quite distinct from the root trace and showed no development of a fibrous cortex, a significant feature, as the dimensions of the two traces were almost the same and their anatomy was not strikingly different. The stele of the bud trace was simple; it consisted of a central core of poorly differentiated

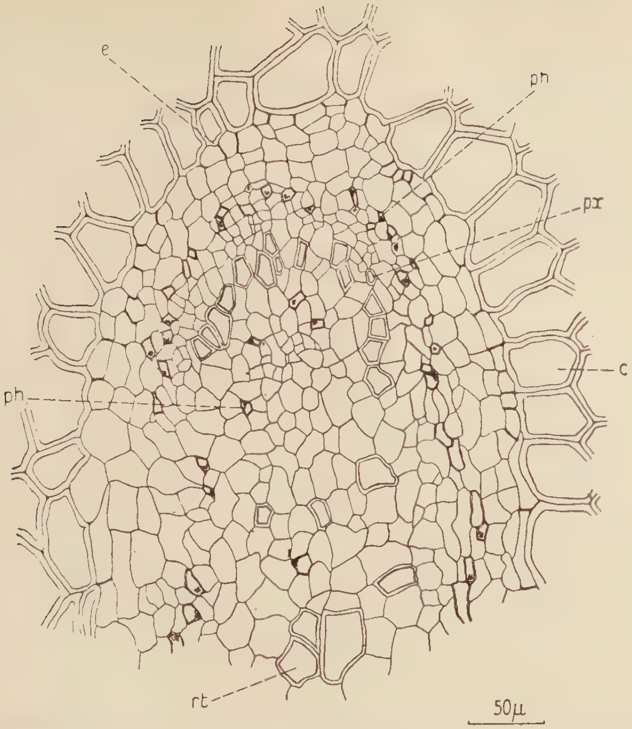

FIG. 6. *Elaphoglossum muscosum*. Drawing of a transverse section of a bud trace at the base of a bud. *c*, cortex of rhizome; *e*, endodermis; *ph*, phloem; *px*, protoxylem; *rt*, root trace.

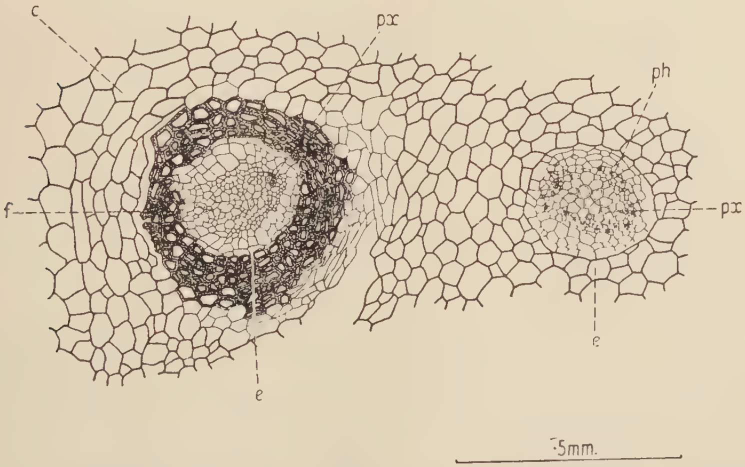

FIG. 7. *Elaphoglossum pallidum*. Drawing of a surface section at the base of a petiole on the abaxial side, showing a transverse section of a bud trace (right) and a root trace arising from it (left). *c*, cortex of rhizome continuous with the cortex of the petiole; *f*, investment of fibrous cells continuous with the fibrous inner cortex of the root; *e*, endodermis; *ph*, phloem; *px*, protoxylem.

xylem, with peripheral protoxylem elements, separated by parenchymatous cells from an ill-defined band of phloem. This was again surrounded by parenchyma and the whole trace bounded by a distinct endodermis. There was a close anatomical resemblance between this trace and a root trace near its insertion on the stele (Fig. 2).

The buds in *E. villosum* were extremely small and did not appear to be always present. In Fig. 4 two buds are seen on the abaxial sides of petioles.

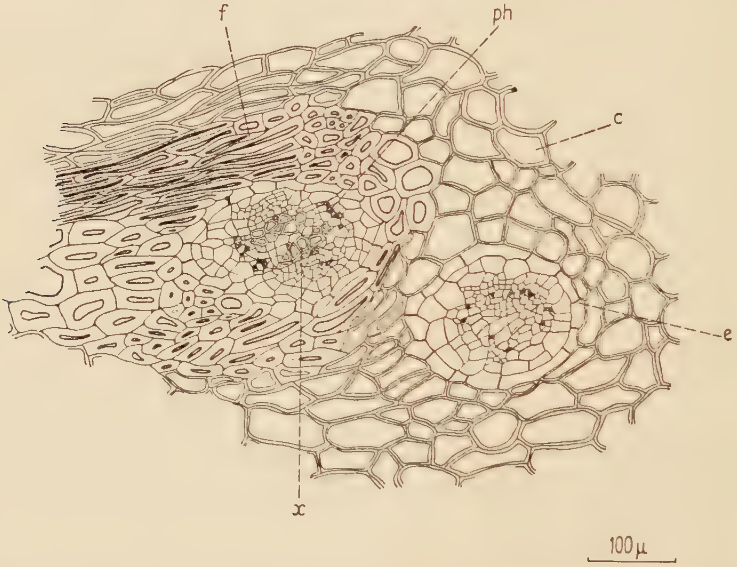

FIG. 8. *Elaphoglossum villosum.* Drawing of a surface section at the base of a petiole on the abaxial side, showing a transverse section of a bud trace (right) and a root trace arising from it (left). *c*, cortex of petiole; *f*, fibrous zone continuous with fibrous inner cortex of root; *e*, endodermis; *ph*, phloem; *x*, xylem.

These buds had the form of a slight elevation surrounding a small central depression. When well developed they might have been taken for abortive roots, but frequently they were so small that their presence could only be conjectured from external observation and had to be verified by sectioning the petiole. A surface section of the abaxial side of a petiole, cutting a bud and an associated root transversely, is shown in Fig. 8. The root was readily identifiable by the zone of fibrous cells, some of which, as the root was running out obliquely, were seen in longitudinal section. The xylem of the root trace was developed normally, except that the central area remained undifferentiated. The neighbouring bud trace was quite distinct and clearly set off from the cortex of the petiole by the endodermis. It showed very much less differentiation than the root trace which had arisen from it.

A central zone of large cells and a surrounding zone of smaller cells probably corresponded to the areas of xylem and phloem.

The relation of the bud trace to the leaf trace. In one series of transverse sections of the rhizome of *E. latifolium* a curious spur of contorted tracheids was observed on the upper anterior surface of the bud trace (Fig. 9). As this series of sections was continued it was found that this tracheidal mass was directed towards another small group of contorted tracheids which apparently arose *de novo* on the abaxial side of the leaf trace. This group of tracheids divided into two arms, one directed downwards which rapidly diminished and disappeared, and another which ascended and fused with a strand linking the median and the lower marginal strands of the leaf trace.

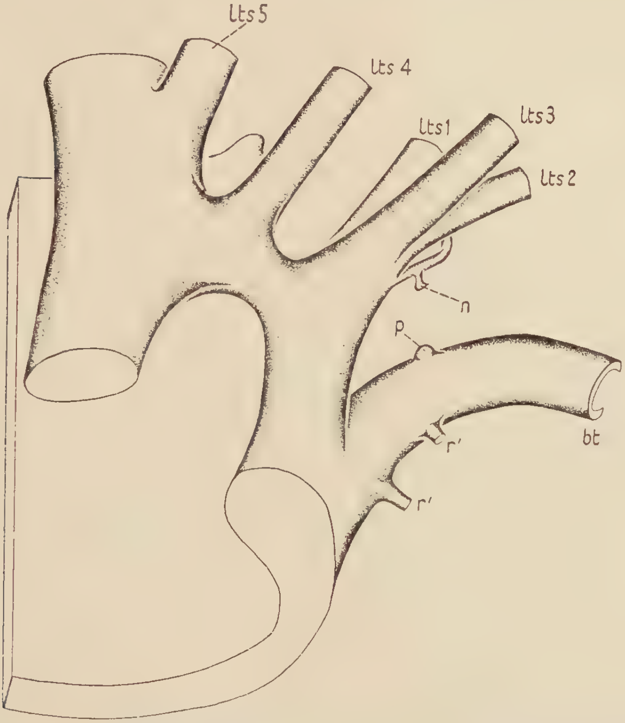

FIG. 9. *Elaphoglossum latifolium.* Reconstruction of part of the stele showing development of vascular tissue between the bud trace and leaf trace. *lts*, strand of leaf trace; *bt*, bud trace; *r′*, root in association with bud trace; *p*, protrusion of tracheids on upper anterior surface of bud trace; *n*, V-shaped group of tracheids, one arm of which unites with a strand connecting a median with the lower strand of the leaf trace.

In another series of sections a group of tracheids arose adjacent to the lowest meristele of the leaf trace (Fig. 10). This group also divided; one arm ascended and fused with the adjacent meristele, the other descended in an obliquely anterior direction and fused with the bud trace.

These structures suggest a tendency for the bud trace to be connected with the leaf trace, but such a connexion is not usual. Frequently neither the bud trace nor the leaf trace show any anomalous structures.

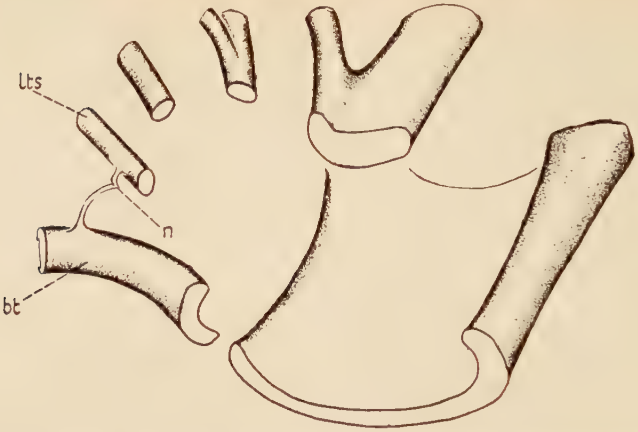

FIG. 10. *Elaphoglossum latifolium.* Reconstruction of part of the stele showing a connexion between the bud trace and the leaf trace. *lts*, strand of leaf trace; *bt*, bud trace; *n*, **V**-shaped group of tracheids, the upper arm of which unites with the lowest strand of the leaf trace and the lower arm with the bud trace.

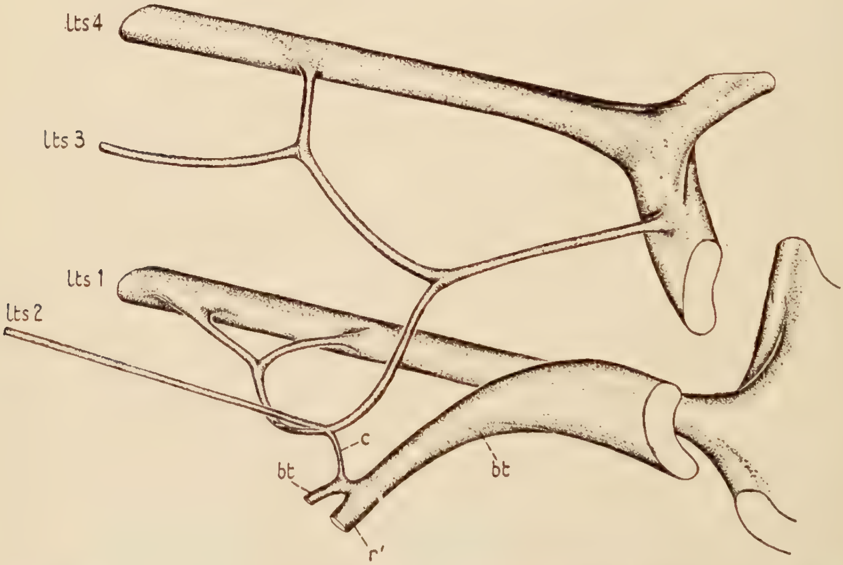

FIG. 11. *Elaphoglossum pallidum.* Reconstruction of the vascular system in the base of a petiole. *lts*, strand of leaf trace; *bt*, bud trace; *r′*, root in association in the bud; *c*, strand connecting bud trace with petiolar system.

No other species with the habit of *E. latifolium* has shown this petiolar system. Further, it has not been observed in either *E. hirtum* or *E. muscosum.*

A very peculiar system was discovered in the petiole of *E. pallidum* (Fig. 11). In this species the bud trace is well developed at its origin but rapidly diminishes in its outward course. In the particular petiole investigated the bud trace gave rise to a root just before its entry into the bud. The development of the root was considerably more than that of the bud, so that the bud trace appeared to be a lateral branch of a main trace ending in the root. Just after the separation of the root and bud traces the bud trace fused with a tenuous strand of tracheids ascending from a plexus of anastomosing strands linking

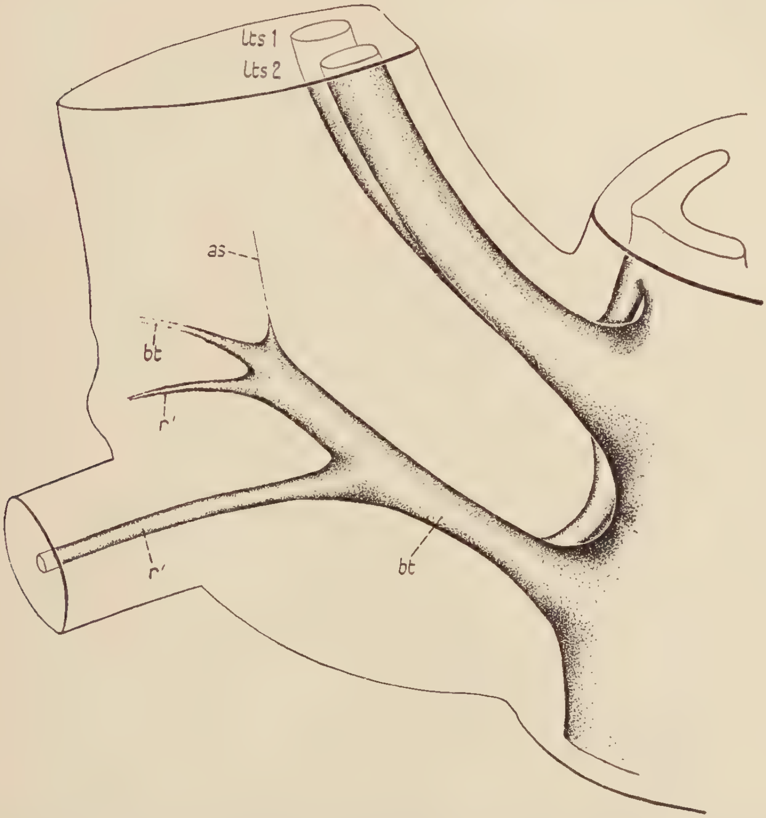

FIG. 12. *Elaphoglossum villosum.* Reconstruction of the vascular system in the base of a petiole. *lts*, strand of leaf trace; *bt*, bud trace; *r′*, root in association with bud; *as*, rudimentary strand ascending towards leaf trace.

the two prominent marginal meristeles of the leaf trace. These anastomosing strands, after the departure of the strand to the bud trace, gave rise to two strands which continued as median meristeles of the leaf trace. No other examples of this petiolar system were discovered in *E. pallidum.*

In *E. villosum* a complex petiolar system was frequently, but not always, found accompanying a bud. The general form of this system was the same in all instances and is shown diagrammatically in Fig. 12. The bud trace arose from the sinus of the leaf gap and passed obliquely outwards, giving rise to one well-developed root trace in its course. The tracheids of the bud trace then became short and contorted and the trace broke up into three, two of which passed outwards and ended in undifferentiated tissue and one which ascended towards the leaf trace, rapidly diminished in extent, and disappeared in a single tracheid.

The morphological interpretation of these traces is based on their anatomy and on a comparison with the preceding species. The lowermost trace of the three acquired a girdle of fibrous cells as it passed outwards and is regarded as belonging to an abortive root. The trace above this showed no fibrous cells, but was separated from the cortex of the petiole solely by an endodermis. This is interpreted as an abortive bud trace. The third strand is regarded as a rudiment indicating a tendency for the bud trace to be connected with the petiolar trace, in a way similar to that in which these two traces are connected in *E. pallidum*.

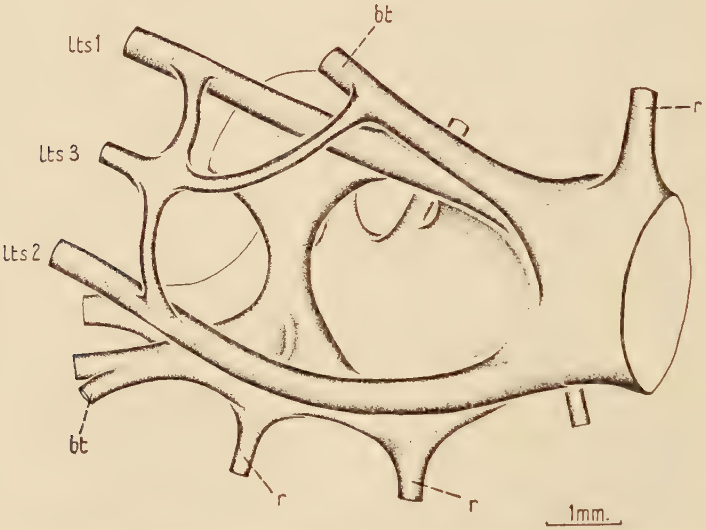

FIG. 13. *Elaphoglossum hirtum.* Reconstruction of the stele of a developing bud. *lts*, leaf trace strand; *bt*, bud trace; *r*, root trace.

(iii) STELAR STRUCTURE IN THE DEVELOPING BUD

The development of the stele was followed in a bud which had grown out to form a short daughter rhizome in a specimen of *E. hirtum*.

The trace entering the bud was protostelic and bore a root trace on its upper surface (Fig. 13). The trace then opened on one side and gave rise on the upper margin of the opening furrow to a poorly developed bud trace ascending towards the upper surface. Immediately anterior to the bud trace, two strands of the leaf trace arose, one from each margin of the furrow, and passed obliquely outwards. These marginal strands of the leaf trace were linked by transverse strands of tracheids with a commissural strand coming from the bud trace. From the fusion of these strands a third ascended into the petiole of the first leaf.

The gap formed by the opening of the stele and the emission of the first leaf trace was closed by a vascular bridge, which was succeeded by another leaf gap and bud trace. Meanwhile, on the other side, a leaf gap developed a little anterior to the first opening of the stele and this again initiated a succession of leaf and bud traces, those of one side alternating with those of the other. One root trace was inserted on the upper surface after the opening of the stele, but, except for this, all root traces arose from the ventral part.

This daughter rhizome had reached about half the diameter of the parent and the fronds borne upon it about one-quarter of the mature size, but, in its immaturity, the stele displayed the bilateral symmetry of the mature stele of *E. latifolium*. The daughter rhizome had not developed sufficiently to show a transition to the mature form of *E. hirtum*, where the fronds are borne in several ranks on the upper surface.

Another piece of rhizome which showed a juvenile form was found in a gathering of *E. muscosum*. This probably represented a daughter rhizome which had developed from a lateral bud and become detached. The fronds were about half the mature size and, in the posterior half of the rhizome, were arranged in two ranks as in *E. latifolium*. In the anterior half of the rhizome the number of fronds increased by the intercalation of additional insertions in the dorsal region. The first appearance of these dorsal insertions was accompanied by a strong curvature of the rhizome away from the upper surface, but it is not known whether this generally occurs. The stelar structure in the anterior part of the rhizome came to assume that of the mature form.

These investigations, which have been limited by the material available, suggest that in *E. hirtum* and *E. muscosum* the lateral bud, in its development to form a daughter rhizome, passes through a phase of symmetry and stelar structure similar to that of the mature form of the bilaterally symmetrical species such as *E. latifolium*. Unfortunately no outgrown buds have been present in material of *E. pallidum* and *E. villosum*.

DISCUSSION

This work has revealed that, in addition to the series of increasing stelar complexity in *Elaphoglossum* described in an earlier paper (Bell, 1950), there is another and parallel series showing progressive reduction in the development of the buds and the bud trace. The bud trace becomes very reduced in *E. pallidum* and *E. villosum*, but the morphological interpretation that is attempted here seems justified by its agreeing with the general pattern of the bud trace seen in the other species described. The interpretation is also supported by the anatomical evidence.

In the older literature there is frequent discussion of whether or not the root in ferns can be gemmiferous at its emergence (Lachmann, 1889). It seems possible from the present work that in some, at least, of those species where the root has been considered gemmiferous, one might be dealing with reduced bud systems. The close proximity of the root to the bud in *E. pallidum* and *E. villosum*, and especially the lateral displacement of the bud by the root in the former, might have suggested the occurrence of gemmiferous roots in this genus, had these species been studied in isolation.

The tendency for the bud trace to be connected with the leaf trace has been detected in only three species, but further observation may show that it is more general, as it is a tendency that appears independently of the degree of development of the bud trace. The significance of this connexion is difficult to appreciate from the species examined hitherto. It may become revealed when it has been possible to examine other species which have a freely branching rhizome and in which the branching bears a definite relation to the position of the leaf.

The ontogeny of the lateral bud in *E. hirtum* and *E. muscosum* is of especial interest, as the view has been frequently held that a developing primordium, foliar or cauline, indicates the phylogeny of the organ in its ontogeny (Bertrand, 1943). The initial bilateral symmetry of the daughter rhizome in these species may have a phylogenetic significance, but it is felt that such speculation should wait until a wider range of species has been investigated.

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Studies in the Genus *Elaphoglossum*

III. Anatomy of the Rhizome and Frond

BY

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With nine Figures in the Text

ABSTRACT

The investigation is continued of a number of species of *Elaphoglossum* from Jamaica.

Features of interest in the anatomy of the rhizome and frond are described. Information, additional to that previously recorded, is given of the scales. It is shown that, in *E. villosum*, complexity of stelar structure and the presence of hydathodes in the fronds is accompanied by scales of a specialized type.

MUCH of the anatomy of *Elaphoglossum* conforms to that known for other ferns. Only features of particular interest or significance are discussed here.

i. ANATOMY OF THE RHIZOME

The unlignified tissues. The ground tissue of the rhizome consists of isodiametric, collenchymatous cells, with the walls perforated by numerous simple pits. Gaps filled with granular material frequently occur at the angles of the cells. There is abundant starch, especially in the ventral region, and tannin cells occur scattered throughout. The outer layers of cells are sclerotic and impregnated with a brown substance, the phlobaphene of the older authors (Walter, 1890). This outer sclerotic zone is not well developed in *E. villosum*.

The vascular bundles. These are embedded in the ground tissue. Each consists of xylem, the first-formed elements of which are peripheral and possess annular and, more rarely, spiral thickening. Parenchymatous cells with dense contents are interspersed amongst the xylem. The phloem consists of clear elongated cells with lateral sieve areas and transverse end walls. The endodermis sharply delimits the meristele from the surrounding ground tissue.

ii. ANATOMY OF THE PETIOLE

External features. The petiole is almost semi-circular in transverse section with a shallow adaxial furrow. In all the nine species examined, with the exception of *E. villosum*, there is a region of disarticulation some 0·5 to 2 cm. above the rhizome. The base of the petiole is black, as is the surface of the

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rhizome, but at the region of disarticulation the colour changes sharply from black to amber and, accompanying the change, is a slight but distinct swelling in the petiole.

Anatomy. The lower region of petiole is covered by an epidermis of isodiametric cells. Within this is a zone of fibrous cells, elongated and prosenchymatous, enclosing the parenchymatous matrix in which the strands of the leaf-trace ascend. At the swollen region the epidermal cells increase in length and remain elongated in all the upper parts of the petiole. The fibrous zone is continuous throughout the swollen region, although there is here, and partly accounting for the swelling, a transient increase in the breadth of this zone.

The walls of the fibrous cells in the lower region are impregnated with phlobaphene and unlignified. Those of the cells above the swelling are lignified, but unpigmented. The transition from pigmented to unpigmented cells in the swollen region is sharp. In *E. hirtum* the fibrous cells at the line of transition are much shorter than elsewhere, but in the other species there are no marked changes in dimensions of these cells at this level. In *E. villosum* the transition from the black area to the amber of the upper petiole is more gradual; there may be a slight swelling, but it is frequently absent and disarticulation takes place close to the rhizome.

The central parenchymatous matrix consists, in the lower region, of almost isodiametric cells with thickened walls, similar to those of the rhizome. In the swollen region the parenchymatous cells become wider and the walls thinner. In the upper part of the petiole the parenchymatous cells are thin-walled and elongated. Here cells containing starch are few, but resin canals occur, especially in association with the vascular bundles.

Disarticulation of the petiole. Contrary to the statement of Mettenius quoted in Christ (1900), no evidence has been found for the presence of a definite abscission layer in the swollen region of the petiole. It is true that in some species, such as *E. latifolium*, very short, broad, cells occur in the parenchyma in the swollen region, but they are not arranged in a definite layer. Also, a line of weakness in the parenchyma alone would hardly account for disarticulation. The tissues possessing tensile strength in the petiole are the fibrous zone and the vascular bundles. In the fibrous zone the sharp transition from the pigmented to the unpigmented cells indicates a metabolic discontinuity and may well provide a line of weakness. Once the fibrous zone was weakened, movement of the frond would cause a great strain to be thrown on the tenuous vascular strands and there would be little to prevent their rupture and the pulling apart of the parenchymatous tissue. In Dicotyledons there is some evidence that short broad cells may appear in the basal regions of petioles and, although they precede, not be the cause of disarticulation (Gawadi and Avery, 1950).

Vascular strands within the petiole. The number of vascular bundles ascending in the petiole varies according to the species and also, within the species, according to the size of the leaf. There are usually three to five strands at the origin, except in *E. villosum* where the number is constantly two. In all species other than *E. villosum* there are frequent oblique anastomoses between the strands. Occasionally, two adjacent bundles may be linked by a bridge of short tracheids springing directly across the intervening parenchyma from one protoxylem group to another. The structure of the bundles shows little variation. There is a central elliptical mass of xylem, with peripheral protoxylem elements, surrounded by parenchyma and phloem, which is more developed on the outside than on the inside of the bundle. The bundle is surrounded by a distinct endodermis. In the base of the petiole only the endodermis separates the bundle from the parenchymatous ground tissue, but in and above the swelling, immediately adjacent to the endodermis, is a girdle of very striking, elongated, asymmetrically thickened cells. The wall of these cells next to the endodermis is very heavily thickened; the thickening diminishes rapidly on the radial wall and is little more than normal on the wall away from the endodermis. The thickening is impregnated with phlobaphene and traversed by numerous simple pits. The lumen of the cell is frequently filled with resin. This girdle surrounds each bundle of the leaf-trace and is present in all species in varying degrees of development. In *E. villosum* there is little or no deposition of the brown pigment, but the cells almost invariably contain resin.

Aerenchymatous tissue in the petiole. In some species, notably *E. chartaceum*, the petiole bears two marginal ridges in line with the decurrent wings of the lamina, and running down to the black area. Here they are usually very obscure, although they may become evident again as the leaf-base joins the rhizome and then gradually diminish and disappear on its surface (*E. latifolium*, Bell, 1950, Fig. 2). In *E. chartaceum* the fibrous zone of the upper petiole is broken on the abaxial side of this ridge by an area of small, rounded parenchymatous cells with large air-spaces. Stomata are found scattered in the epidermis over this aerenchyma. All the species showed some development of this tissue below the lamina.

iii. ANATOMY OF THE LAMINA

Venation. The petiole is continued through the lamina as a prominent midrib reaching to the apex of the frond. Fine lateral veins depart from the midrib at a fairly wide angle, in some species almost at a right angle, and run parallel to the margin. Lateral veins may occasionally dichotomize and the branches run parallel. The veins either end before the margin in a slightly swollen group of contorted tracheids or are linked by a commissural strand at the margin (e.g. in *E. latifolium*, Fig. 1) which may or may not be continuous (Bower, 1928). In *E. pallidum* the swollen ends of the lateral veins are frequently bifurcate, but the arms of adjacent bifurcations are not linked. In *E. villosum* the lateral veins end in hydathodes opening on to the upper surface of the lamina (Fig. 2).

The number of vascular strands becomes reduced in the petiole so that only two or three enter the midrib, still surrounded by the conspicuous girdle cells.

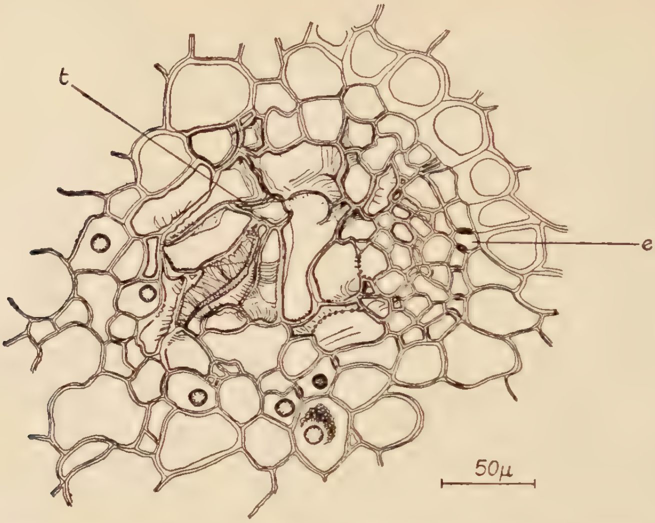

FIG. 1. *Elaphoglossum latifolium.* Drawing of a transverse section of a strand linking the lateral veins before the margin of the lamina; *e*, endodermis; *t*, tracheidal cells.

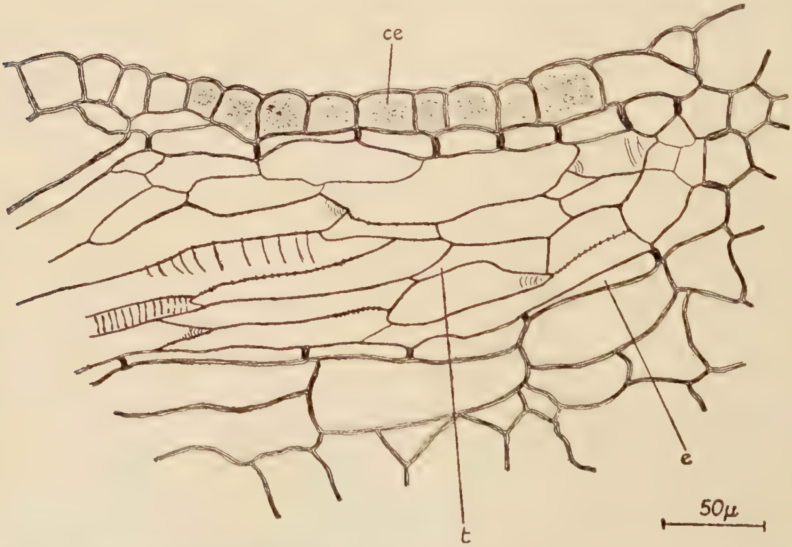

FIG. 2. *Elaphoglossum villosum.* Drawing of a section of a hydathode in the longitudinal plane of the entering nerve; *ce*, cubical epidermis over the hydathode; *e*, endodermis; *t*, tracheidal cells.

Where there are three strands the lateral veins arise only from the marginal strands; the median third strand forms anastomoses with the other two and eventually vanishes. Near the apex the two marginal strands combine and this single strand ends in contorted tracheids in the apex.

Texture of the lamina. With the exception of *E. villosum*, where it is thin, lax, and translucent, the lamina in all species is fairly firm and in some, e.g. *E. latifolium*, coriaceous, with a narrow cartilaginous border. In *E. pallidum* and *E. villosum* the apex of the frond is often attenuated into a 'drip tip'. In all species, again excepting *E. villosum*, there is a well-developed cuticle on the upper surface of the lamina.

The epidermis and stomata. The cells of the upper epidermis have sinuose walls, but the walls tend to be less sinuose over the veins. In *E. muscosum* the walls of the epidermal cells are incrassate and porose. In *E. pallidum* and *E. villosum* the walls are less closely, but more deeply, sinuose. Stomata are confined to the lower surface and are arranged with their long axes more or less parallel to the lateral veins. Comparative estimates of their frequency were made on mature fronds of the different species. *E. tectum* and *E. Eggersii*, in both of which the lamina is scaly on the lower surface, showed the highest frequencies. *E. villosum* showed the lowest frequency and other species, in which the mature frond is glabrous or sparsely scaly below, were intermediate. Stomatal size showed very little change throughout. In the material available there was a suggestion that the stomata of *E. muscosum* tended to be more circular in outline than those of other species. The stomata show a varying relationship to the mother cell. In *E. latifolium* (Fig. 3 A) and in most other species the stomata are usually surrounded by two or more epidermal cells. In *E. villosum*, on the other hand, each stoma lies within an epidermal cell, the wall of which becomes adnate to that of the guard-cells at one end of the stoma (Fig. 3 B). There is a tendency for the stomata to be in this position in *E. pallidum*.

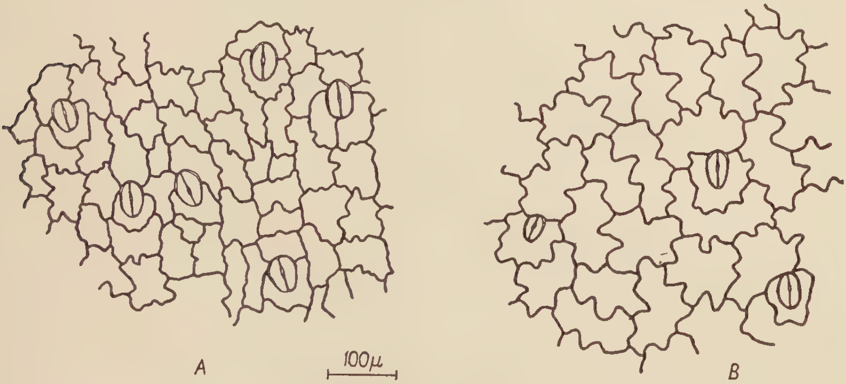

FIG. 3. A, *Elaphoglossum latifolium*; B, *E. villosum*. Stomata on under surface of lamina.

Structure of the lamina. The epidermal cells are chlorenchymatous. Beneath the epidermis in *E. latifolium* is a clearly marked palisade layer, beneath which is spongy mesophyll. In other species the palisade layer is not clearly marked; the cells are all more or less isodiametric, but the uppermost layer has few air-spaces. In *E. villosum* there are only one to two layers of mesophyll and the air-spaces are particularly large. The lateral veins run in the mesophyll.

They consist of a few tracheids with marginal protoxylem elements; there is a little phloem on the abaxial side and a few surrounding parenchymatous cells with dense contents. The endodermis is well developed and surrounding the bundle is a girdle of chlorenchymatous cells, the inner walls of which, adjacent to the endodermis, are heavily thickened with cellulose. Where there is a commissural strand linking the ends of the veins, as in *E. latifolium*, this strand consists of contorted tracheidal cells (Fig. 1). A few parenchymatous cells are present, but no recognizable phloem. There is a discontinuous endodermis.

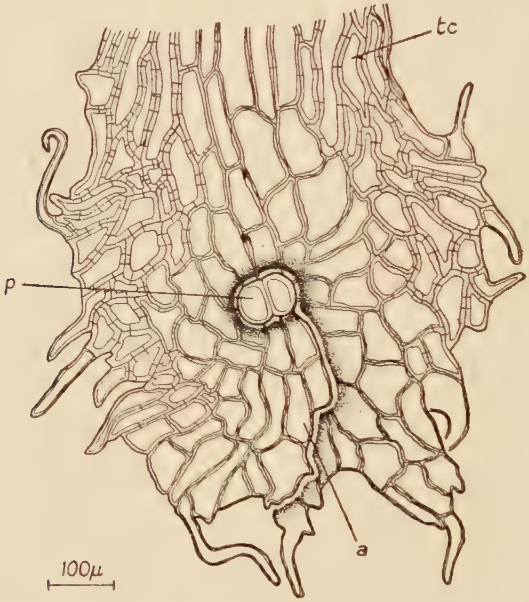

FIG. 4. *Elaphoglossum tectum.* Base of scale from rhizome; *tc*, incrassate cells of upper part of scale; *a*, overlapping auricle of base; *p*, pedicel of scale.

The hydathode of E. villosum. The structure of this organ is shown in Fig. 2. The epidermal cells over the hydathode are cubical and filled with a fine granular or globular material. Immediately beneath these is the endodermis, which is continuous around the whole structure. The tracheidal cells become shorter and more numerous in the hydathode; they are surrounded by a few thin-walled parenchymatous cells, but no phloem tissue. This structure appears to be similar to that of hydathodes recorded in several species of *Polypodium* and other tropical ferns (Poirault, 1893).

IV. SCALES OF THE RHIZOME AND FROND

The scales play an important part in the systematics of the genus and they have been well described by Christ (1900). The details of form and structure presented here are supplementary to Christ's account.

Scales of the rhizome. All the specimens possessed scales on the rhizome, but in one species, *E. Sellowianum*, they were too necrotic to examine their structure and no other material was available. The scales of the rhizome are from a few millimetres to a centimetre in length, depending upon the species. They are ovate-lanceolate in shape, frequently with ciliate or glandular margins. The scales are never more than one cell thick and the cells are elongated with sinuose walls. In *E. tectum* (Fig. 4) the walls of the cells of the upper part of the scale are very heavily thickened, pigmented, and porose, but in other species the thickening is not marked. The attachment of the scale is, in most species, clearly basal, but in *E. tectum* and *E. chartaceum* the scale is deeply cordate and auriculate at the base and the auricles are widely and closely overlapping, so that the attachment appears, at first sight, set in from the margin. The auricles are also decurrent on the pedicel by which the scale is inserted in the epidermis of the rhizome.

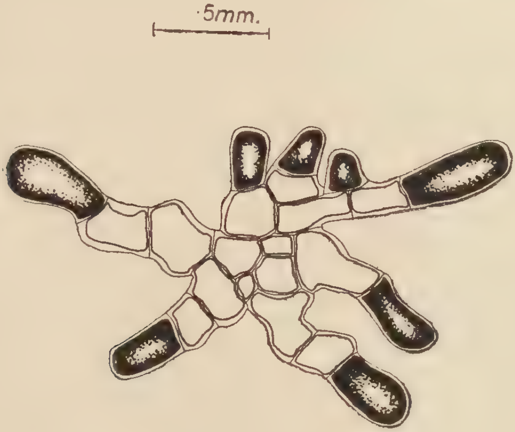

FIG. 5. *Elaphoglossum latifolium.* Scale from surface of young frond.

Scales of the petiole and lamina. In all the species examined, with the exception of *E. villosum*, where they are almost perpendicular to the surface, the scales are appressed to the surface of the frond. The following features, unrecorded by Christ, have been observed.

E. latifolium. On both surfaces of the lamina of the immature frond are very small glandular scales (Fig. 5). These scales are lost from the upper surface of the mature frond, but persist scattered on the lower surface. The attachment appears quite superficial and the scales brush off without leaving a scar. They are similar to the scales of the young petiole, but are more completely divided up into glandular processes.

E. Sellowianum. Broadly ovate-lanceolate scales, 4 to 5 mm. long, occur on the basal 4 to 5 cm. of the petiole; above, at least in mature specimens, the petiole is naked. The margin of the scale is irregular and bears scattered cilia, especially towards the base. The base is cordate and the sinus closed, with the lobes overlapping. The upper surface of the lamina bears minute scales which are almost entirely divided up into filiform processes (Fig. 6). These scales are inserted by a basal pedicel into a small pit, so that, in the mature frond, the scale little more than closes the mouth of the pit. Similar scales, a little larger in size, occur on the lower surface of the frond. Scales of the petiolar type occur scattered along the midrib at the back of the frond.

E. chartaceum. Small scales with glandular processes similar to those of *E. latifolium* are present on the petiole and lamina.

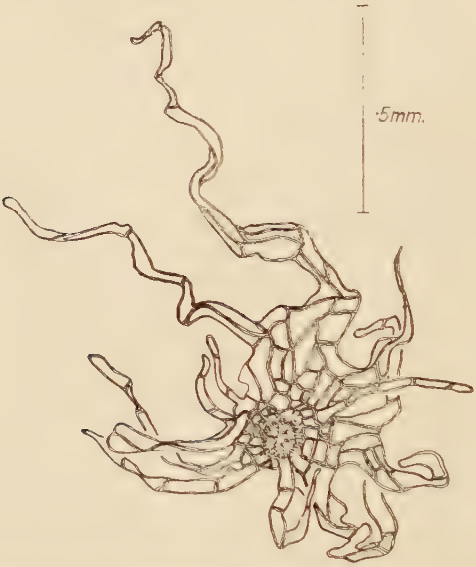

FIG. 6. *Elaphoglossum Sellowianum.* Scale from upper surface of frond.

E. muscosum. The scales vary considerably in size, but are all similar in form. The base is cordate, but the sinus is widely open, and the attachment is by a pedicel at the base of the sinus. In some of the scales on the lower surface of the lamina the margins of the sinus run down vertically on to the pedicel.

E. hirtum. The scales of the petiole have a cordate base, the margins of which are decurrent on to the pedicel. Similar scales occur on the lamina of the frond. In addition there are, on the upper surface, scales with the lamina reduced to only one or two cells, so that the structure resembles a stellate hair. The pedicel is inserted at the base of a small pit which is surrounded by an elevated epidermal ring.

E. pallidum. The frond lacks scales when mature, but both the petiole and the lamina are closely beset with simple and branched glandular hairs (Fig. 7).

E. villosum. The upper part of the petiole and both surfaces of the lamina bear, in addition to simple glandular hairs, arising from epidermal cells, scattered, bristle-like scales. These scales are very narrowly lanceolate with

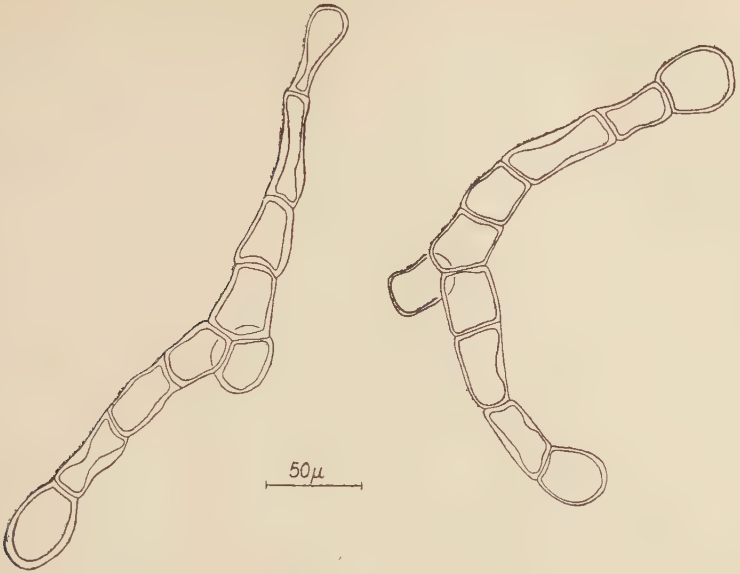

FIG. 7. *Elaphoglossum pallidum.* Glandular hairs from lower surface of frond.

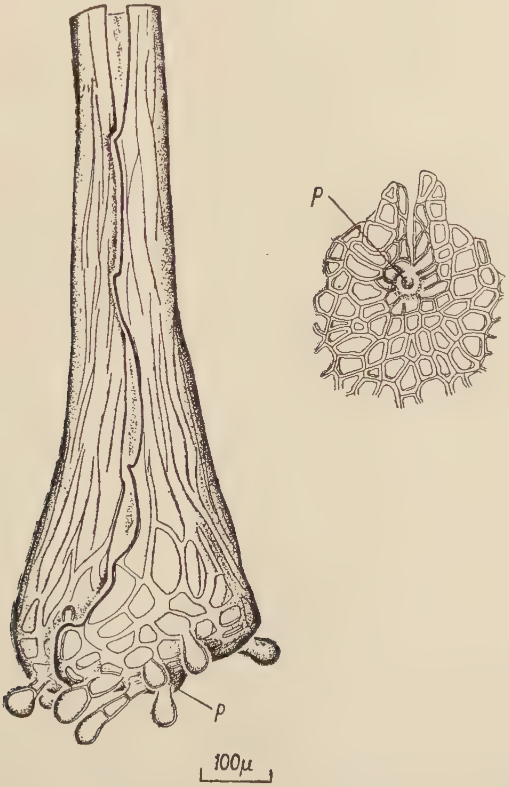

FIG. 8. *Elaphoglossum villosum.* Basal part of scale from lamina; *p*, pedicel.

the margins toothed and widely involute, so that the whole scale becomes more or less cylindrical (Fig. 8). The base is deeply cochleariform and auriculate, the base and the auricles being glandular and closely applied to the surface of the lamina. The cells of the upper part of the scale are long, narrow, and thin walled, those of the base isodiametric and incrassate. The attachment is from the base of the sinus and consists of a short pedicel, on to which the margins of the sinus are decurrent, inserted into a pit in the surface of the lamina (Fig. 9). The pedicel is two-celled at its base and sclerotic. The attachment is immediately above the mesophyll, so that the cells of the base of the pedicel do not form part of the epidermal layer.

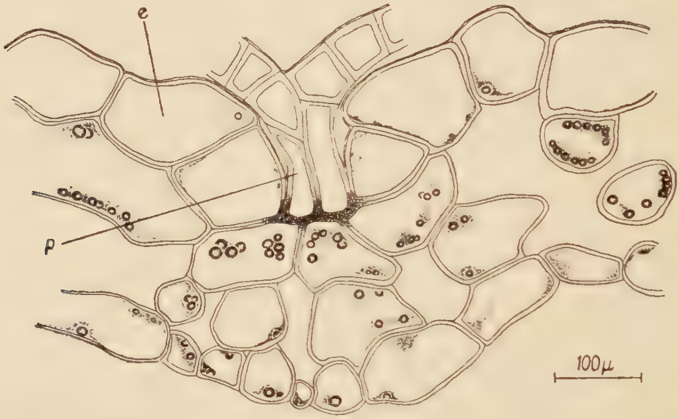

FIG. 9. *Elaphoglossum villosum.* Drawing of a transverse section of the lamina showing the attachment of a scale; *e*, upper epidermis of lamina; *p*, pedicel of scale.

General morphology of the scales. The complexity of the dermal scales in this genus has impressed previous authors attempting to determine its phylogenetic status (Bower, 1928; Christ, 1900). The essential form of the scale within the Jamaican species studied here is a unistratose, ovate-lanceolate structure. The basal region may be cordate and auriculate, and to this can be related the more specialized scale of *E. villosum*. When the base of the scale is simple, the pedicel is usually attached to the lamina superficially. Increasing complexity of the scale is accompanied by the development of a sclerotic pit in the epidermis, at the base of which the pedicel is attached.

V. CONCLUSIONS

The features of possible phylogenetic significance which emerge from a comparative study of the anatomy of Jamaican species of *Elaphoglossum* are:

(*a*) The sharp difference, in the species with horizontal creeping rhizomes, between the parenchyma of the rhizome and that of the petiole and frond. In *E. villosum* the difference is less marked and the transition more gradual.

(*b*) The conspicuous swelling in the petiole in *E. latifolium* and similar species where the change from the rhizome-like anatomy to that of the upper petiole takes place. This swelling is much less conspicuous in *E. pallidum* and is hardly present in *E. villosum.*

(*c*) The tendency for the deposition of phlobaphene in the rhizome and in the cells surrounding the vascular bundles in the frond in those species with creeping rhizomes. This substance is much less evident in *E. villosum.*

(*d*) The difference in position of the stomata in relation to the epidermal cells between *E. villosum* and the other species examined. The relationship in *E. pallidum* approaches that in *E. villosum.*

(*e*) The occurrence of hydathodes in the lamina of *E. villosum* and the presence in this species of peculiarly complex scales.

An evaluation of the significance of these facts awaits the accumulation of further evidence.

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Studies on Plankton Parasites

III. Examples of the Interaction between Parasitism and other Factors determining the Growth of Diatoms

BY

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With Plates XVI and XVII and four Figures in the Text

ABSTRACT

This paper deals with the interrelations of populations of plankton organisms and their parasites in two English lakes. The organisms considered are diatoms, mainly *Asterionella formosa* but also *Tabellaria fenestrata* var. *asterionelloides*, *Fragilaria crotonensis*, and *Melosira italica*, with a blue-green *Oscillatoria agardhii* var. *isothrix*. The parasites are the Chytrids *Rhizophidium planktonicum* on diatoms and *R. megarrhizum* on *Oscillatoria*.

The parasites may delay the time of maximum algal number or may decrease the size of the maximum. Generally parasitism of one alga favours the development of others. Various other complexities arise from the interaction of these factors with nutritional conditions.

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INTRODUCTION

THE periodicity of the diatom *Asterionella formosa* Hass in the plankton of certain lakes in the English Lake District has been studied for a number of years (Canter and Lund, 1948; Lund, 1949, 1950). Parasitism is only one of the factors controlling the changes in the density of the population from time to time. The effects of parasitism on the further development of the host population do not only depend on the growth[1] rate of the parasite relative to that of the host but also on chemical, physical, and biological

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[1] The term 'growth' is here used for the increase in numbers of a population and not for any increase in cellular dimensions.

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factors, the interactions with some of which are here illustrated. The present account refers to populations in the water column from the surface to 5-m. depth. This is representative of all depths under isothermal conditions and usually of the epilimnion when the water is thermally stratified. Moreover, the latter is usually representative of the photic zone in the lakes concerned, light being insufficient for growth in the hypolimnion (Lund, 1949, Fig. 6, and pp. 405–9).

The course of a parasite epidemic is, briefly, as follows (for details see Canter and Lund, 1948). Encysted fungal zoospores are observed in rapidly increasing numbers on the diatom cells which are otherwise quite healthy in appearance (compare Pl. XVI, A, and Pl. XVII, A); the numbers of well-developed or empty sporangia and dead or dying diatoms are low. The ratio of sporangia to encysted zoospores now rises, as does that of dead or dying to live cells (Pl. XVI, B). Finally, a large part of the population bears sporangia, most of which are empty, and dead diatom cells abound (Pl. XVII, B). During this last stage sexually formed fungal resting-cells may be observed.

METHODS

The methods of collection and estimation of the host and parasite populations are described in Canter and Lund (1948) and Lund (1949, 1950). While the numbers of *Asterionella*, *Tabellaria fenestrata* (Lyngb.) Kütz var. *asterionelloides* Grun., and *Fragilaria crotonensis* (Edw.) Kitton are estimated as cells, those of *Oscillatoria agardhii* Gom. var. *isothrix* Skuja are estimated as filaments per unit volume.

Time did not permit counting as many colonies of *Fragilaria crotonensis* as of *Asterionella* in order to obtain a mean value for cells per colony. Whereas colonies of *Asterionella* usually contain between 4 and 8 and very rarely over 16 cells, those of *Fragilaria* usually contain between 30 and 60 and not uncommonly between 100 and 200 live cells. In 1949 the mean of 50 and in 1950 the mean of 20, sometimes 50, colonies was taken. For this reason, the confidence limits for the density per millilitre of *Fragilaria* are wider than for *Asterionella*. This applies particularly to low densities (e.g. 20 cells or less per ml.). The major changes in density are, however, clearly shown. Other methods of estimation, such as the length of the filament, are wholly untrustworthy because of the variations in the length of the pervalvar axis and the variable number of dead cells present.

Previously (Canter and Lund, 1948) the infection was recorded as the percentage of diatom cells bearing viable parasite cells (e.g. encysted zoospores or undehisced sporangia). In the following the percentage of diatoms bearing empty sporangia is also included. The former is called the percentage of the population bearing *infective* cells and the latter that bearing *infected* cells. The high percentage of infection often recorded at the end of an epidemic is largely due to the large number of diatom cells bearing empty sporangia. The method does, however, give a correct picture of the severity of an epidemic.

WINDERMERE, SOUTH BASIN, AUTUMN 1948 AND 1949

The north and south basins of Windermere are separated by an area of shallow water containing several islands and, from a biological point of view, the south basin may be considered as a separate lake with the north basin water as its main inflow (Mill, 1895; Lund, 1949, 1950). It also receives the outflow of Esthwaite Water. Collections were made at a fixed buoy in the northern portion of this basin. The depth at this point is 35 m.

In this lake *Asterionella* has two periods of abundance, spring and autumn. The autumnal is less than the vernal maximum because, though the nutrient supply is generally sufficient during it and the winter minimum which follows, the growth rate of the diatom becomes progressively less as winter approaches and illumination decreases. The depth into which illumination sufficient for growth passes becomes progressively less, while in the upper layers of the water, where it is sufficient, the amount received per day also decreases (cf. Lund, 1949, Fig. 7). Temperature changes reinforce this, for reduced water temperature reduces the rate of growth (Lund, 1949, Fig. 10); it also leads to the loss of the summer thermal stratification of the water. During thermal stratification cells in the upper layers are more or less permanently located there since the thermocline separates them from the lower layers (where light is insufficient for growth) by the zone of temperature discontinuity (thermocline). With the loss of thermal stratification and complete mixing of the lake water by wind action, the majority of the cells at any one time are in layers where light is insufficient for growth. If severe parasitism occurs in the early stages of the autumnal period of diatom increase, it will reduce the actual maximum by keeping the numbers low at a time when the growth rate is greatest.

Fig. 1 shows that, in September 1948, the numbers of *Asterionella* fell sharply (from over 150 to less than 5 per ml.) when a severe fungal epidemic occurred. At the time of maximum infection more than 90 per cent. of the cells were parasitized by *Rhizophidium planktonicum* Canter (for a description of this fungus see Canter and Lund (1948); it is possibly an aggregate species). In the absence of this parasitism the numbers of *Asterionella* might have been expected to increase, as is suggested by the dotted curve which continues from the point marking the observed maximum population (September 8). This curve is based on the rate of increase observed at this place during this period in 1945 when no fungal epidemics or other events, such as floods, leading to marked losses of cells occurred. However, if conditions had permitted such an increase, a further factor limiting the maximum production would have come into play, namely, depletion of available silica. Ten million cells of *Asterionella* contain approximately 1·4 mg. of silica, the amount of silica per cell being apparently a specific character unaffected by environmental conditions (Einsele and Grim, 1938; Lund, 1950). The theoretical maximum in the absence of parasitism (dotted line) represents such a population per litre, but at no time was the concentration of dissolved silica in the

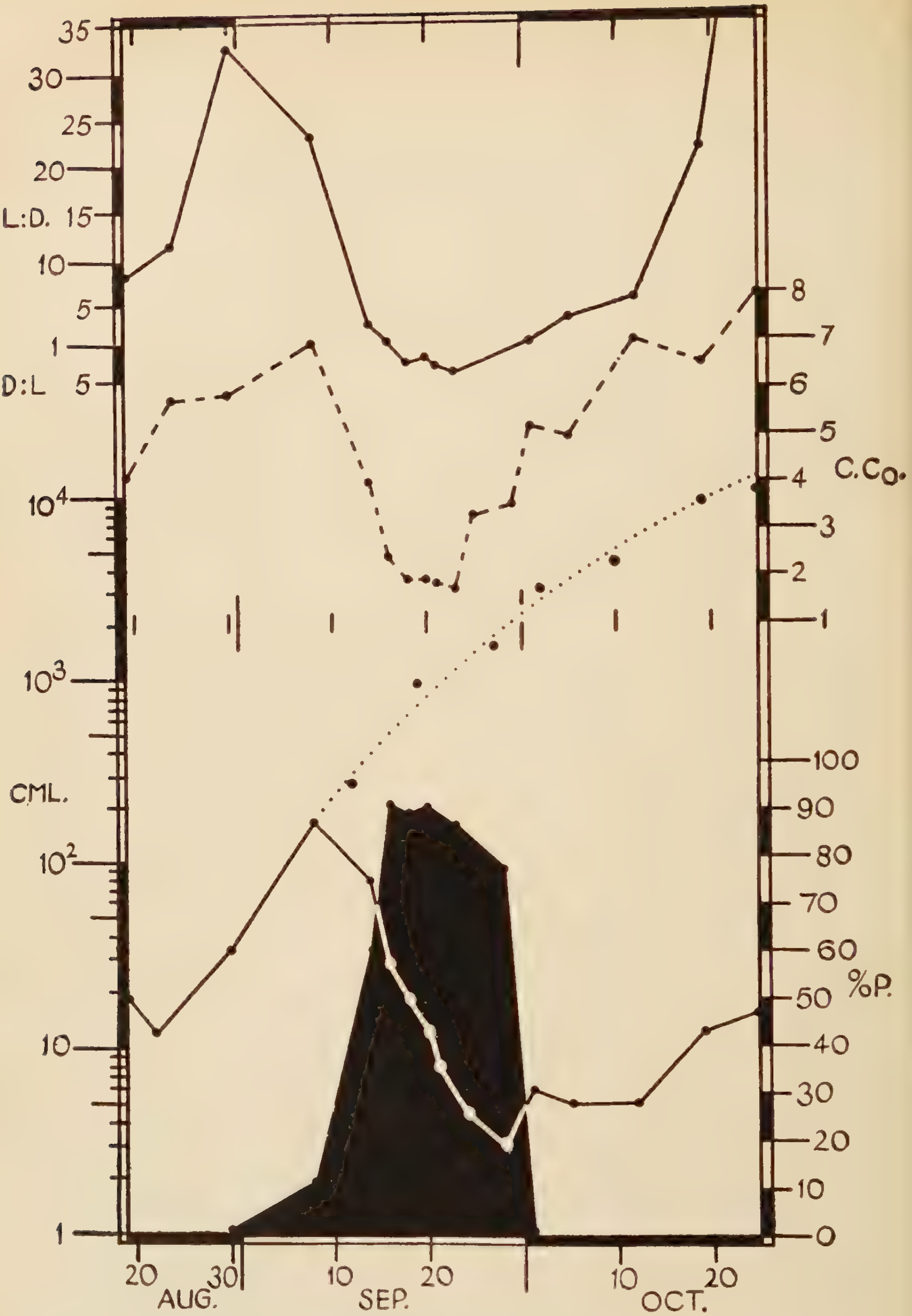

Fig. 1. Parasitism of *Asterionella formosa* in relation to light penetration, water temperature, and nutrient depletion. Windermere, south basin, autumn 1948. Upper continuous line: proportion of live to dead cells in the population (L:D and D:L); interrupted line: number of live cells per colony (C.CO); lower continuous line: number of live cells per ml. (C.ML.) plotted on a logarithmic scale; dotted line: theoretical number of cells which might have been produced in the absence of parasitism (see text); solid black: percentage of *Asterionella* cells infected by *Rhizophidium planktonicum* (% P).

water sufficient to produce more than 6·5 million cells so that the increase in numbers would have ended on or before October 15. It might well have ended before October 15 since other diatoms would probably have used at least a part of the silica.

The graph also shows that during the course of a fungal epidemic the number of live cells per colony of *Asterionella* (interrupted line) decreases as does the proportion of live to dead cells in the population (uppermost continuous line). With the end of the epidemic the reverse series of changes occurs, though the ratio of live to dead cells remains low longer than the number of live cells per colony. This difference is due to the presence of single dead cells produced by the disintegration of the colonies killed by parasitism; these take some time to sink or be lost by outflow. A more prolonged (October to December) but less severe epidemic occurred in the autumn of 1949 and only a small maximum (48 cells per ml.) of *Asterionella* was reached.

ESTHWAITE WATER, SPRING 1949 AND 1950

Collections were made at a fixed buoy at the north end of the lake in the area of maximum depth (15 m.).

The usual course of events in the spring is as follows. With increasing light (about the first or second week in February) the rate of increase of *Asterionella* becomes greater than the rate of loss (to outflow, bottom, &c.). The density of the population rises until all the available silica has been used in the second half of April (Lund, 1950). By the middle of May very few live cells are left. When the supply of silica is replenished from the inflows growth may be renewed for a short time but no large population is produced. Some time after thermal stratification of the water sets in, usually June or July, some factor other than the silica supply limits the growth of *Asterionella*, and usually all other plankton diatoms, so that growth is not renewed until September or October. Such a cycle of events occurred in 1945, 1946, 1947, and 1948 (Lund, 1950).

Three other diatoms are present in lesser numbers. *Melosira italica* (Ehr) Kütz reaches its maximum before any large utilization of silica has occurred. *Tabellaria fenestrata* var. *asterionelloides* has a similar periodicity to *Asterionella* but sometimes shows a decline in growth rate or even a decrease in numbers before the numbers of *Asterionella* are limited by lack of silica. *Fragilaria crotonensis* commonly starts its spring increase later than *Asterionella*. All the diatoms concerned are unable to produce high densities when the concentration of silica in the water falls to 0·5 mg. per litre. Since the estimation of silica is only correct to about 0·1 mg. per litre, the limiting concentration for diatom growth shown in the graph (Fig. 2) lies between 0·6 and 0·4 mg. per litre. *Oscillatoria agardhii* Gom. var. *isothrix* Skuja[1] was never abundant during the period under review in the years 1945 to 1948 inclusive.

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[1] This species (Skuja, 1948, p. 49) has been recorded from Windermere and Esthwaite Water under diverse names by previous authors.

In 1949 (Fig. 2) a very slow increase or even at times a decrease in numbers occurred during the period of rapid increase of the previous years, nor was the concentration of dissolved silica in the water markedly reduced (Lund, 1950, Fig. 1). Not till the second week in May did the typical rapid rise in numbers start, and then it only lasted about a fortnight before a catastrophic drop in numbers occurred. This atypical periodicity may be related to three other factors not operative in the previous years. Firstly two fungal epidemics on *Asterionella*, the first, unlike most epidemics (Canter and Lund, 1948), lasting about 2 months; secondly the abundance of *Oscillatoria agardhii* var. *isothrix*; and thirdly a period of heavy loss to the outflow due to floods in April. Observations and experiments to be described elsewhere show that a large growth of *Oscillatoria* (*circa* 10^5 filaments per litre) reduces the light penetration to such an extent that the growth rate of *Asterionella* is signi-

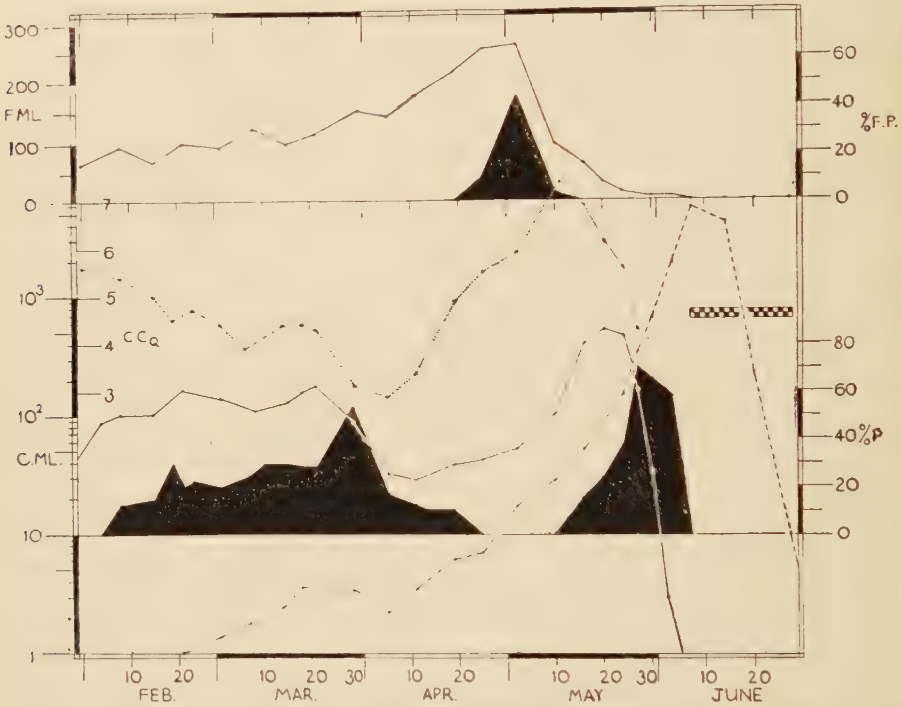

FIG. 2. Growth and parasitism of *Asterionella formosa* in relation to physical and chemical conditions and the abundance of other algae. Esthwaite, spring 1949. Upper continuous line: number of *Oscillatoria agardhii* var. *isothrix* per ml. (F.ML); upper solid black: percentage of *Oscillatoria* filaments infected by *Rhizophidium megarrhizum* (% F.P); lower continuous line: number of *Asterionella* cells per ml. (C.ML), plotted on a logarithmic scale; interrupted line: number of *Fragilaria crotonensis* and *Tabellaria fenestrata* var. *asterionelloides* cells combined (C.ML) plotted on the same logarithmic scale; lower solid black: percentage of *Asterionella* cells infected by *Rhizophidium planktonicum* (% P); dotted line: number of live cells per *Asterionella* colony (C.CO); the black and white grid marks the period when there was insufficient available silica for diatom growth.

ficantly reduced. The course of events was then as follows. The start of the normal spring increase of *Asterionella* was delayed by the growth of *Oscillatoria* and the prolonged fungal epidemic which started in the second week in February. Towards the end of March parasitism reached a peak (54 per cent.) and this was followed by the period of high rainfall. The numbers of *Asterionella* which had remained more or less static now fell. With the end of the flood period came also the end of the first fungal epidemic. The numbers of *Oscillatoria* filaments, however, increased sharply, and though the numbers of *Asterionella* increased, the rate of growth was slow. The next change came when the *Oscillatoria* filaments were themselves parasitized by *Rhizophidium megarrhizum* Sparrow (see Appendix). This fungus has a long rhizoidal system which permeates through and kills as many as 70 cells of the host. The numbers of *Oscillatoria* now declined rapidly. With this change a period of sunny weather occurred and the numbers of *Asterionella*, now almost free of parasites, rose rapidly. In the middle of May, however, there was a second period of severe parasitism of *Asterionella*, shorter but more severe than the first epidemic. The rate of increase of *Asterionella* then declined and was followed by an increasingly rapid rate of decrease in numbers. To understand the last change it is necessary to consider the changes in the numbers of the plankton diatoms, *Tabellaria fenestrata* var. *asterionelloides* and *Fragilaria crotonensis*, together with their utilization of the available silica in the water. During both the periods when *Asterionella* was severely parasitized the numbers of these two diatoms were increasing, relatively slowly at first[1] and, after the *Oscillatoria* growth decreased, as rapidly as *Asterionella*. Neither of these diatoms was severely parasitized at any time (though *Fragilaria* at other times has been even more severely parasitized than *Asterionella*), and from being present in lesser numbers they became dominant during the second epidemic on *Asterionella*. Finally they reached such a density that the silica concentration in the water fell below 0·5 mg. per litre, when their growth too was halted. There was then insufficient silica for any of the plankton diatoms and so a renewal of growth of *Asterionella* was also impossible and all three diatoms rapidly declined to minimal densities. Fig. 2 also shows the changes in the mean number of live cells per colony in relation to the fungal epidemics.

In 1950 (Fig. 3) also no large maximum of *Asterionella* occurred, the causes being apparently in part similar to those of 1949 and in part different. From the third week in January to the second week of April a third or more of the population was parasitized. In addition there were floods in February, while *Oscillatoria* was abundant from the second week in March to the second week in May. As in 1949, the numbers of *Asterionella* either increased slowly or remained more or less static while the fungal epidemic lasted. *Tabellaria fenestrata* var. *asterionelloides*, on the other hand, increased steadily until the second week in April; its rate of growth was, however, relatively slow owing possibly to the floods in February and abundance of *Oscillatoria* afterwards. *Fragilaria crotonensis* was present in small numbers during this period. Neither of these two algae bore more than a very few parasites. After the parasite epidemic ended, the numbers of *Asterionella* did not increase even slowly as in 1949. Further, those of *Tabellaria* at first remained static and then decreased while those of *Fragilaria* remained static until May. The cause of this difference from 1949 is unknown, but it would appear to be due to some effect similar to or the same as that which keeps the numbers of these diatoms low in most years after the spring maximum even though there is an ample supply of silica (Lund, 1950). Indeed, the silica concentration never fell below 0·6 mg. per litre in the period under review. In June the numbers of *Asterionella* and *Tabellaria* fell to very low levels, while those of *Fragilaria* after a short period of increase in May and June likewise decreased. The changes cannot be attributed to the abundance of *Oscillatoria*, since in 1949 even greater densities did not preclude a slow rate of increase of the plankton diatoms.

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[1] It should be remembered that the estimations of low numbers of *Fragilaria* are very approximate.

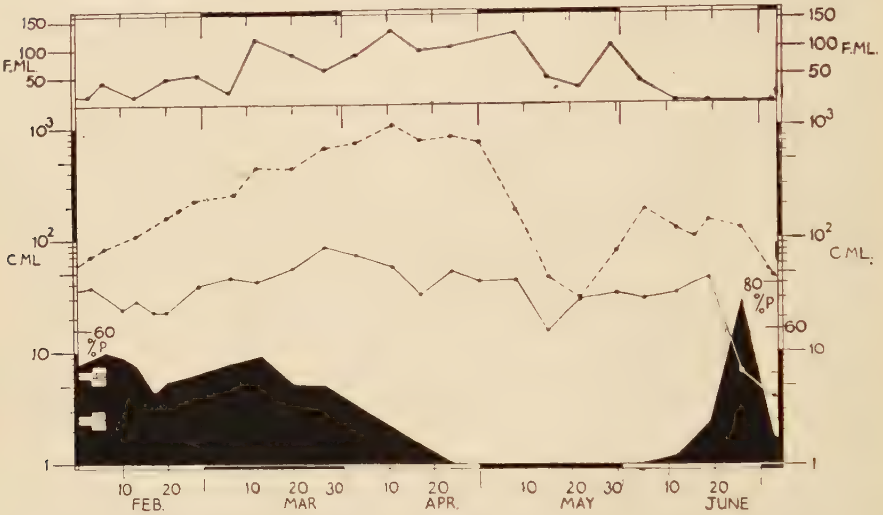

Fig. 3. Growth and parasitism of *Asterionella formosa* in relation to physical and chemical conditions and the abundance of other algae. Esthwaite, spring 1950. Upper continuous line: number of filaments of *Oscillatoria agardhii* var. *isothrix* (F.ML); lower continuous line: number of *Asterionella* cells per ml. (C.ML) plotted on a logarithmic scale; interrupted line: number of *Fragilaria crotonensis* and *Tabellaria fenestrata* var. *asterionelloides* cells combined (C.ML) plotted on the same logarithmic scale; solid black: percentage of *Asterionella* cells infected by *Rhizophidium planktonicum* (% P).

SUMMARIZING DISCUSSION

It is now possible to draw some conclusions concerning the interrelations of the factors acting on *Asterionella* and the competing plankton diatoms with special reference to parasitism.

Parasitism may delay the time of the maximum; it may also decrease its size. If the physical and chemical conditions remain suitable, the effect will be almost wholly one of delay. There may be a relatively small reduction in the maximal density of live cells due to the loss of a nutrient such as silica in the form of dead frustules. There will be no loss in the total (dead and live) of cells produced. If conditions change during or a relatively short time after an epidemic, the size of the maximum may be reduced. A change in physical conditions (e.g. light) brought this about in Windermere, south basin, in 1948 and 1949. An unknown change in the chemical conditions brought it about in Esthwaite in the spring of 1950. In Esthwaite, in 1949, the diatom maximum as a whole was similar in size to that of previous years because other diatoms, themselves relatively free of parasites, took the place of *Asterionella*. In 1950 conditions became unfavourable for the growth of all the planktonic diatoms.

Parasitism of one alga may favour the development of other algae. Esthwaite 1949 provides an example for competing diatoms. Moreover, the parasitism of the blue-green alga *Oscillatoria* increased the rate of growth of all the diatoms and enabled them to reach a high density before chemical conditions other than the supply of silica became limiting.

Other things being equal at the time of the maximum, dominance of species with similar demands on the environment will depend on the relative size of the populations at the start of the period of increase. This is generally the case with *Asterionella formosa*, *Tabellaria fenestrata* var. *asterionelloides*, and *Fragilaria crotonensis* which start their spring period of increase at more or less the same time (*Fragilaria* usually somewhat later than the other two) and reach a maximum when the silica supply is exhausted. *Melosira italica* is an exception to this in that its cycle of development is not wholly synchronous with these three species. Its maximum is normally reached in the latter half of March before the silica supply is exhausted. Observations elsewhere show that, if other conditions are favourable, it can continue growth until the silica concentration in the water falls to 0·5 mg. per litre as is the case with the other diatoms.

The observed degree of infection of the algal population depends on the relative growth rates of host and parasite. However, if conditions are suitable, the parasite can grow faster than *Asterionella* can at any time in the lakes concerned. This is shown to be the case by the fact that epidemics may occur at any time of year (Canter and Lund, 1948) and be preceded or followed (Fig. 2) by rapid growth of *Asterionella*. If a hyperparasite of the fungus concerned also occurs (see Appendix), the relative growth rates of the two fungi and the alga will determine the severity of an epidemic.

Occasionally the occurrence of an epidemic may coincide more or less closely with the period when the host population is about to decline from other causes (e.g. Canter and Lund (1948), p. 250, Fig. 7). This appears to be the case for the parasitism of *Oscillatoria* in Esthwaite Water in 1949 (Fig. 2), for no renewed increase in numbers occurred after the epidemic ceased.

Even in such cases, however, an epidemic may exert an appreciable ecological effect since the time of the decline of the host population is then earlier than it would otherwise have been, or the decrease in numbers is hastened so that they rapidly fall to levels below which they exert little or no effect on the other algae in the plankton. In Esthwaite in 1949, if the numbers of *Oscillatoria* had declined more slowly, it is possible that the available silica in the water might not have been utilized by *Tabellaria* and *Fragilaria* before some other factor reduced their rate of increase as occurred in 1950 (Fig. 3).

More than one factor may be adversely affecting the growth of an alga during one period. It is then difficult or impossible to separate the effects of these factors. The observable effect of one may mask the effect of the others. This was the case in Esthwaite Water in 1950, when a factor other than parasitism limited the growth of *Asterionella*, either during part of the period when it was severely parasitized or directly afterwards. However, it is possible to infer that the action of this unknown factor only started during the closing phases of the epidemic, for it was then that the growth rate of the other plankton diatoms decreased, at first slowly and then rapidly. In 1949, by contrast, it is reasonable to infer that no such unknown factor came into play. In that year, when the parasite epidemic on *Asterionella* ended, the growth rate increased, slowly at first and fast after the growth of *Oscillatoria* declined (Fig. 2). Moreover, the other plankton diatoms increased slowly or fast throughout the whole period under review until the supply of silica was exhausted. Further indirect evidence for this view is supplied by the number of live cells per colony of *Asterionella*, which showed a rapid rise after the end of the first fungal epidemic. While the number of cells per colony is not invariably a sound criterion of the vigour of the diatom, it is usually the case that high numbers indicate a capacity for fast growth if the physical conditions (e.g. light) permit.

APPENDIX

The Chytrid Parasite of Oscillatoria agardhii Gom. *var.* isothrix Skuja

Rhizophidium megarrhizum Sparrow (1936) occurs occasionally in the English Lake District lakes, Esthwaite Water, Windermere (South Basin), and Loughrigg Tarn. Professor C. T. Ingold (personal communication) reports it on *Oscillatoria* sp. in Bradbourne Park Lake, Riverhead, Sevenoaks, Kent. It occurred on *Oscillatoria rubescens* DC. in Zürichsee, Switzerland, in July and August 1949.[1] In contrast to most other Chytridiaceous fungi infecting plankton algae, we have been able to grow it on its host for several weeks in the laboratory, where the samples (from Esthwaite Water) were illuminated, aerated, and kept cool.

Dangeard (1886), De Wildeman (1890), and Sparrow (1936, 1943) state that the zoospore usually settles on the apex of the *Oscillatoria* trichome. More than one may do so (Fig. 4, B–G), the largest number observed in

[1] Sample kindly sent by Dr. E. A. Thomas.

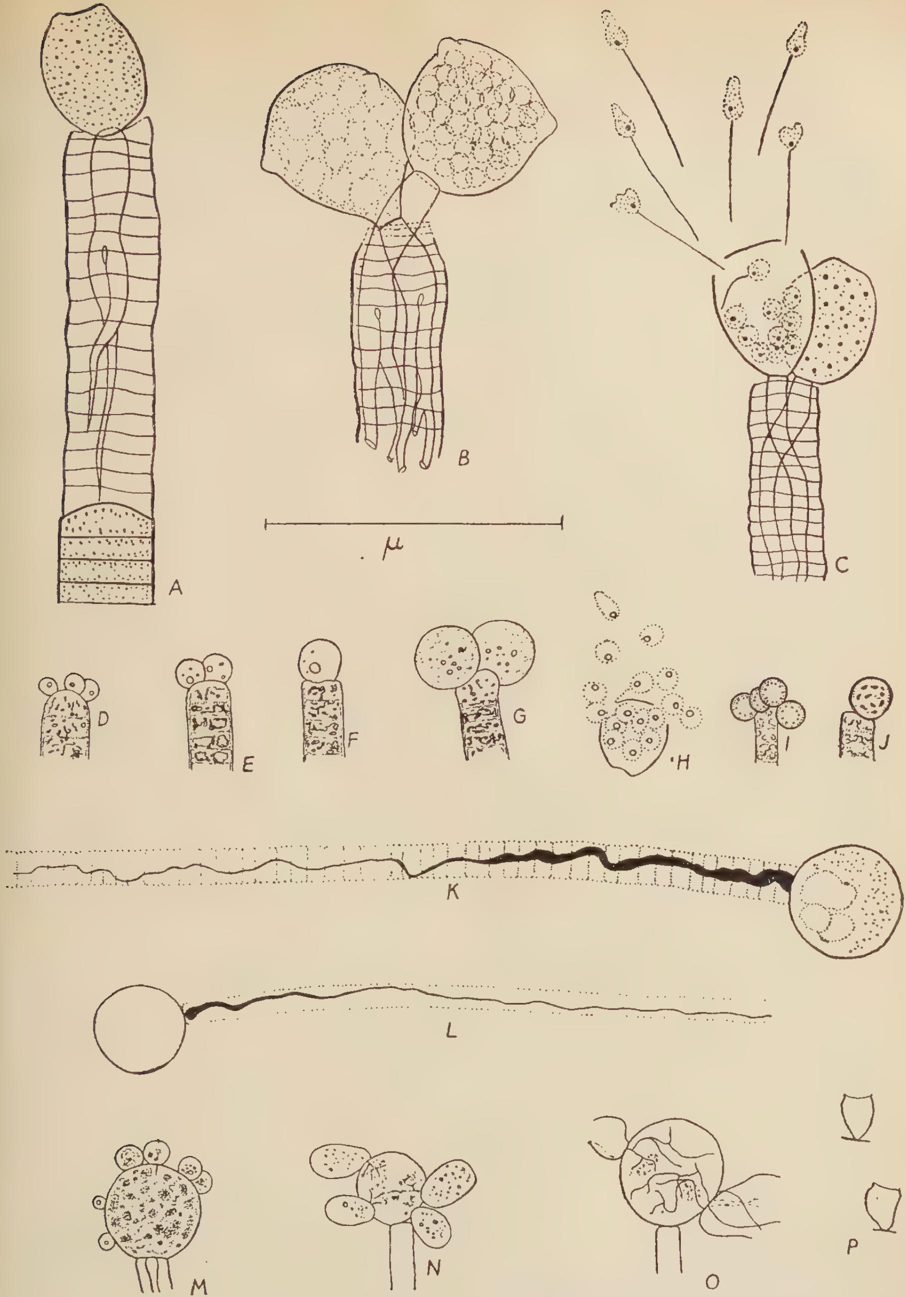

FIG. 4. *Rhizophidium megarrhizum* Sparrow. A–C on *Oscillatoria* sp. from Bradbourne Park; D–K on *Oscillatoria agardhii* var. *isothrix* from the English Lake District; D encysted zoospores on apex of trichome; E–G, immature sporangia; H, dehiscing sporangium; I, J, resting spores; K, sporangium and rhizoid; L as K but on *Oscillatoria rubescens* from Zürichsee, Switzerland; M–P, hyperparasite on *Rhizophidium megarrhizum* from Zürichsee; M, encysted zoospores and very young sporangia; N, immature sporangia; O, empty sporangia with branched rhizoidal system; P, two empty sporangia. A–C drawn by Professor C. T. Ingold, the rest original. A–C as scale below B; D–H, J, M–P, ×800; I ×500; K ×1100; L ×500.

samples from the English Lake District being ten. Infection may also occur at a point of fracture of the trichome. The sporangium (Pl. XVII C; Fig. 4, B, C, K, L) is at first spherical (6–14 μ in diameter), but the formation of the dehiscence papillae (1 to 3) may make the ripe sporangium pyriform or angular. The zoospores were never motile before deliquescence of these papillae. They emerge singly, three or four in rapid succession at irregular intervals. The free-swimming zoospore (2·5–3·5 μ diam.) is spherical with a minute basal refractive granule and single posterior flagellum. The delicate wall of the empty sporangium crumples soon after dehiscence is completed.

The rhizoid is depicted as tubular, relatively short, and sometimes forked by Ingold (Fig. 4, A, B), Sparrow (1936, Pl. 17, Figs. 1 and 2), and De Wildeman (1890, Fig. 5). In the English and Swiss material it is longer and never forked (Pl. XVII C, Fig. 4, K, L), most nearly resembling that depicted by Dangeard (1886, Pl. 13, Fig. 3). Only the broad apical portion is visible in live material, but staining with aceto-carmine shows that it follows a long meandering course (up to 118 μ long) through the trichome, killing up to 70 cells. It tapers distally. Resting spores (Fig. 4, I, J) occurred in the material from the English Lake District, mixed with sporangia and during the later stages of a period of infection. The resting spore appears to be asexual. It is extra-matrical, spherical (5·4–9 μ diam.), and small relative to the larger sporangia. The wall is thick and smooth; the content oleaginous and refractive. No rhizoidal system has been seen.

The figures of other workers show that, as described above, two types of rhizoid occur. The differences may be related to the varying widths of the trichomes of the *Oscillatoria* species infected (Table I). Table I shows that

TABLE I

Recorded Dimensions of Rhizophidium megarrhizum. *All measurements in μ*

	Breadth of alga.	Breadth of rhizoid at apex.	Length of rhizoid.	Size of sporangia.	Zoospore.
Sparrow . .	15	5	60–5	15–16	3
Ingold . .	11	5	48	13–18 br.; 17–18 h.	3
De Wildeman* .	10	4·2	50	—	—
Switzerland .	6–8	3	150	14–22 br.; 15·5–23 h.	2·8
Lake District .	5·4–6·6	2·3–3·6	118	6–14	2·5–3·5
Dangeard . .	5·1†	1·7†	84†	15–18	3
Minden . .	6–8†	1·8†–2·8†	65–78†	?–20–25 br.;	2·5

* De Wildeman (1890) gives no magnification for his figures and no dimensions for the fungus. However, presuming the zoospores drawn by him are 3 μ and the figures are all at the same magnification, the breadth of the algal filament and length of the rhizoid can be determined. It seems likely that both these assumptions are correct since the zoospore is recorded by all other workers as 3 μ and the *Oscillatoria* threads drawn by De Wildeman are all of the same width.

† These measurements are only approximations, being based on Dangeard's (1886) or Minden's (1911) figures.

all the recorded dimensions of the zoospores and sporangia are similar and there seems no reason to suppose that two fungi are involved.

In the Swiss material this fungus was itself infected by a hyperparasite which cannot yet be named (Fig. 4, M–P). It is clear that hyperparasitism may also exert an important effect on the interactions between a fungal parasite and its host. If it occurs in large enough numbers and at an early enough stage in an epidemic, the reduction of the numbers of the algal host will be lessened and the course of the epidemic more or less markedly altered. No information on this point is available for the English material.

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EXPLANATION OF PLATES

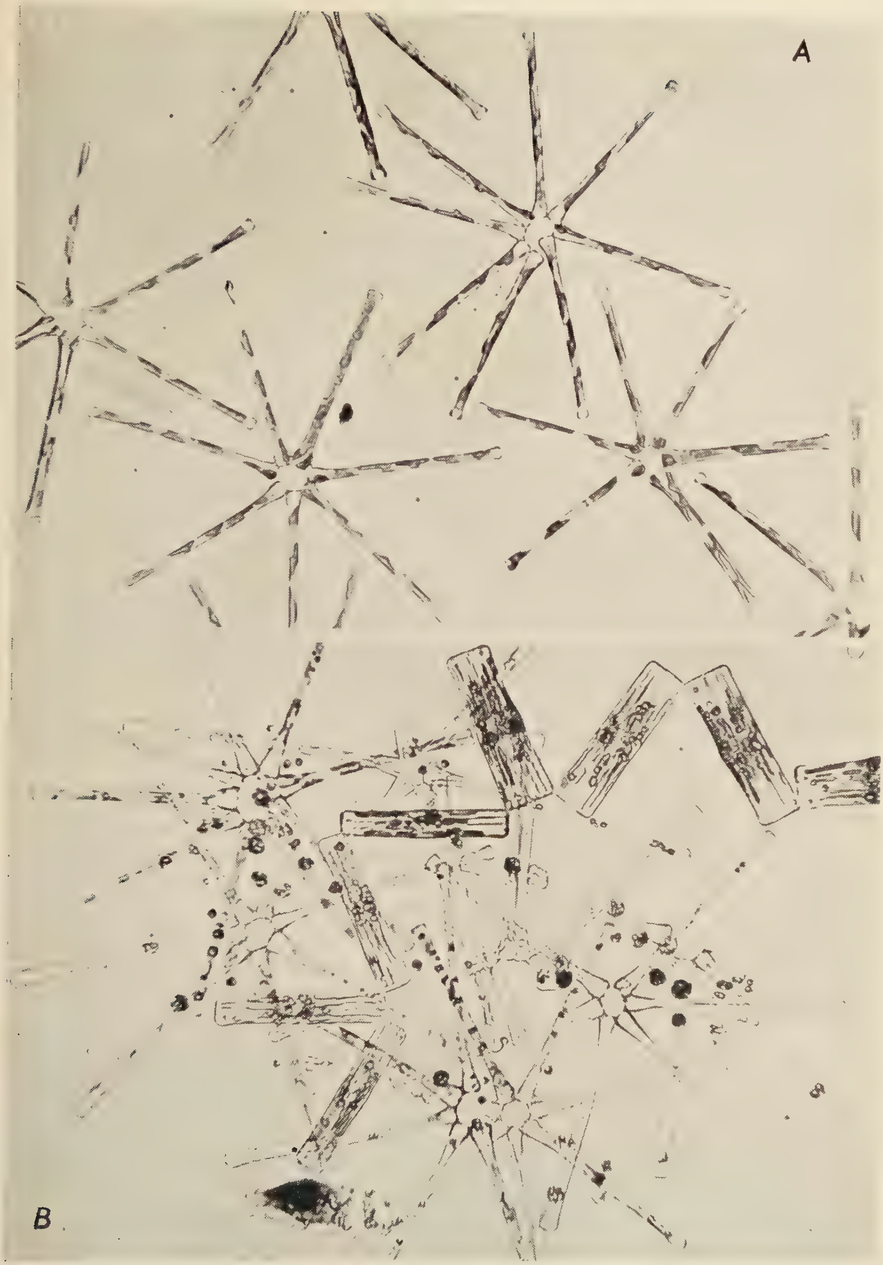
Illustrating H. M. Canter's and J. W. G. Lund's article on 'Studies on Plankton Parasites III. Examples of the Interaction between Parasitism and other Factors determining the Growth of Diatoms'.

PLATE XVI

A, part of a healthy, and B, part of a heavily parasitized, population of *Asterionella formosa*. In B note numerous sporangia of *Rhizophidium planktonicum* and uninfected colony of *Tabellaria*. Both × 500.

PLATE XVII

A, colony of *Asterionella formosa*, two infected cells each bearing an encysted zoospore; host cells not visibly different from the five other uninfected cells. B, colony bearing several empty sporangia of *Rhizophidium planktonicum* and disorganized remnants of the chromatophores of the host cells remaining. C, filament of *Oscillatoria agardhii* var. *isothrix* infected by *Rhizophidium megarrhizum* Sparrow; stained in acetocarmine to show long tapering rhizoid traversing many host cells. A, B × 740; C × 940.



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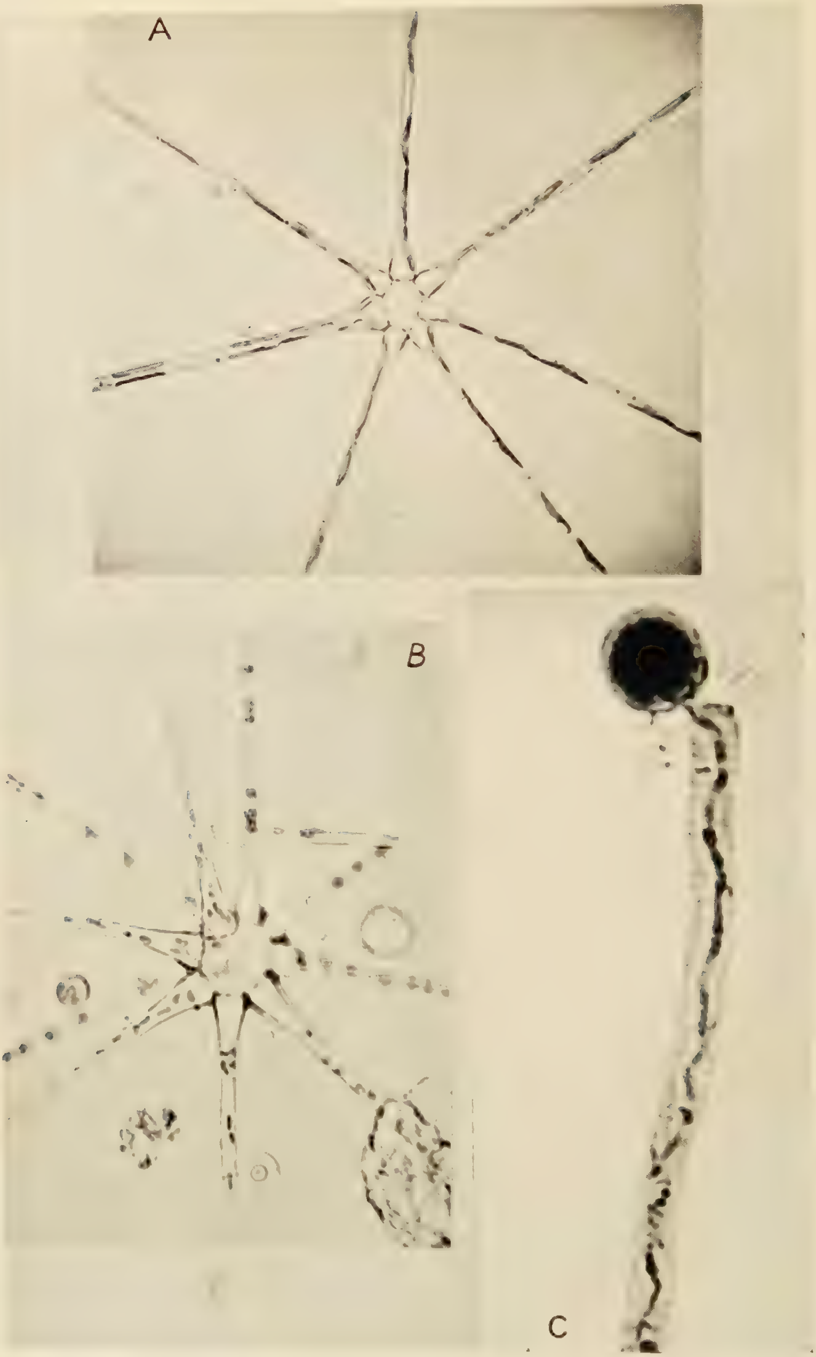

H. M. CANTER AND J. W. G. LUND

Physiological and Ecological Studies in the Analysis of Plant Environment

VII. An Analysis of the Differential Effects of Light Intensity on the Net Assimilation Rate, Leaf-Area Ratio, and Relative Growth Rate of Different Species

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With twenty-four Figures in the Text

ABSTRACT

Since relative growth rate is the product of net assimilation rate and leaf-area ratio (leaf area/plant weight), it follows that if the effects of shading on both net assimilation rate and leaf-area ratio can be expressed mathematically, then the relationship between light intensity and relative growth rate can be derived from the product of the two mathematical expressions.

For all the ten species investigated in field and pot culture experiments, it has been found that during the early vegetative phase both the changes in leaf-area ratio and net assimilation rate, over the range of 0·1 to full daylight, are linearly related to the logarithm of the light intensity. In consequence, the relationship between relative growth rate and the logarithm of light intensity—being the product of the two linear regressions—is curvilinear.

For species of shady habitats (*Geum urbanum*, *Solanum dulcamara*) neither the levels of assimilation rate nor the 'compensation-point' values are very different from those of the eight species from open situations (e.g. *Hordeum vulgare*, *Pisum sativum*, *Fagopyrum esculentum*). Nevertheless the intensity at which growth rate is maximal varies between species: it is 0·5 for *G. urbanum*, 0·7 for *H. annuus*, full daylight for *F. esculentum*, while for *Trifolium subterraneum* the calculated value is 1·8 daylight. Such specific differences can be largely accounted for in terms of the differences in leaf-area ratio at the different light levels.

On the basis of this analysis of the light factor, a 'shade' plant is best redefined as a species in which a reduction of the light intensity causes a rapid rise in the leaf-area ratio from an initial low value in full daylight: for a 'sun' plant the converse definition holds.

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INTRODUCTION

NET assimilation rate was first defined by Gregory (1917) as the rate of increase in total plant weight per unit of assimilating material, e.g. leaf-area. Subsequently Briggs, Kidd, and West (1920) pointed out that the 'relative growth rate'—equivalent to the 'efficiency index' of Blackman (1919)—was the product of the net assimilation rate and the ratio of total leaf area to total plant weight, which they termed 'leaf-area ratio'. In other words, at any instant

$$\frac{1}{W}\frac{dW}{dt} = \frac{1}{dt}\frac{dW}{A} \times \frac{A}{W},$$

where W = total plant weight and A = total leaf area. Over a period of time between two sampling occasions (t_1 and t_2) Blackman (1919) showed that the mean growth rate could be expressed as

$$\frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}.$$

Similarly the net assimilation rate can be calculated from the equation

$$\frac{W_2 - W_1}{t_2 - t_1} \times \frac{\log_e A_2 - \log_e A_1}{A_2 - A_1}$$

provided that total plant weight is linearly related to total leaf area (Williams, 1946). Again with this same proviso the mean leaf-area ratio can be expressed as the arithmetic mean of the values for the two sampling occasions.

The previous papers of this series have been primarily concerned with the effects of reduced light intensity on the growth and development of *Scilla non-scripta*, and it was first established for this species (with plants grown in the open) that over the range of 0·1 to full daylight the net assimilation rate is linearly related to the logarithm of the light intensity, whereas the relationship between relative growth rate and light intensity is curvilinear (Blackman and Rutter, 1948).

Subsequently the investigations have been extended to cover the effects of shading on many other species of higher plants, and it has again been found (Blackman and Wilson, 1950) that this linear relationship between net assimilation rate and the logarithm of the light intensity holds for ten other species as long as the environmental conditions at the highest light intensities permit reasonable growth. The present paper contains a further assessment of the effects of varying light intensity on these ten species, especially the interrelationship between net assimilation rate, leaf-area ratio and relative growth rate.

Since the relative growth rate is a product of the net assimilation rate and the leaf-area ratio, it follows that the effects of light intensity on the relative growth rate can be interpreted in terms of net assimilation rate and leaf-area ratio once their relationships with light intensity have been determined. Just as the previous papers established that the data relating net assimilation rate with the logarithm of light intensity could be fitted with precision by linear regressions, the present paper will demonstrate that a similar logarithmic relationship exists for light intensity and leaf-area ratio. In consequence, the quadratic equations obtained by multiplying the pairs of linear regression equations for net assimilation rate and leaf-area ratio accurately express the changes in relative growth rate brought about by shading.

The extent and scope of the present investigation has been given in some detail in the preceding paper (Blackman and Wilson, 1950). Briefly, between 1942 and 1945 field experiments were conducted at Slough, while between 1947 and 1948 pot experiments of very similar design were undertaken at Oxford. The principal object of the Slough experiments was to assess at different times in the year the effects of shading on the growth and development of *Helianthus annuus*. The research at Oxford has sought to amplify some of the Slough findings and to extend the investigations to cover nine other species, namely, *Hordeum vulgare*, *Vicia faba*, *Pisum sativum*, *Fagopyrum esculentum*, *Lycopersicum esculentum*, *Tropaeolum majus*, *Trifolium subterraneum*, and two species associated with shady habitats—*Geum urbanum* and *Solanum dulcamara*.

EXPERIMENTAL METHODS

The design, experimental methods, and techniques employed in these investigations have been fully set out in the previous paper (Blackman and

Wilson, 1950); in consequence, only an outline will be given here. At both Slough and Oxford all the experiments were of a random block design, with at least four replications. At Slough the site of the experiments was an open field with an infertile gravel soil; a split plot design was employed in which half of each plot received additional nitrogen, phosphorus, and potassium. At Oxford the experiments were confined to pot culture trials, and in order to ensure that there was no deficiency of the major nutrients a complete fertilizer was added to the pots.

In the field experiments sunflower seed was drilled in rows 30 cm. apart and immediately after emergence the plants were singled to 15 cm. apart in the row. In each pot experiment, according to the species and the experiment, the plants were thinned to a standard number of 4–7 per pot of 25-cm. diameter.

The number of light intensities was standardized at four in the field experiments, but in the pot trials the number ranged from three to five. The maximum light intensity was always natural daylight and the reductions in intensity were achieved by suspending in a horizontal plane above the plants light wooden frames covered either with butter muslin or sheets of zinc perforated with a varying number and size of holes.

For the estimation of the degree of shading cast by the screens the methods of Atkins, Poole, and Stanbury (1937) with some modifications were employed. Either two Weston or two Evans photo-electric cells, covered with flashed opal glass, were connected by a two-way switch to an ammeter. One cell was then put in the open in a horizontal position and the other under the screen, level with the tops of the plants, and with the two-way switch almost simultaneous pairs of readings were obtained. Since in neither instance were the cells precisely matched, they were recalibrated for each set of readings.

In order to obtain comparable results between experiments the plots were first sampled and the screens put in position when the plants had passed the seedling stage and had reached the phase of rapid growth. As far as possible a standard size was selected for all species. For example, with *H. annuus*, *P. sativum*, and *F. esculentum* the initial sampling occasion took place when the second pair of true leaves had started to expand and the third pair could be clearly seen. The plants were next sampled when it was judged they had at least doubled their weight in full daylight.

For the field experiments, at each sampling occasion eight plants were chosen at random from each split plot, dug up carefully with a trowel, transported to the laboratory, and the soil washed from the roots. The plants were then divided into root, leaves, and shoot, a subsample of the leaves taken for the determination of leaf area, and all samples dried at 100° C. and weighed. A very similar procedure was adopted in the pot experiments. The main differences were firstly that the finer roots were recovered from the pots by washing the soil through sieves, and secondly that the leaf petioles were included with the stem weight, so that the leaf sample consisted solely of laminae.

Two somewhat similar methods were used for the estimation of leaf area. For the Slough experiments the leaves were placed between two sheets of glass, illuminated from below, and the outlines drawn on uniform sheets of paper. The weights of the cut-out paper leaves, a square of paper measuring 100 cm.^{2} from the same sheet, and the dry weight of the leaf subsample were then determined. In the Oxford experiments the leaves of the subsample were placed on photo-sensitive paper, pressed down with a sheet of glass, and 'blue-printed'. Subsequently the area was measured by a planimeter, while the leaf subsample was dried and weighed. From either set of figures the ratio of the area of fresh leaf per gramme dry weight was calculated for each leaf subsample, and this factor applied to the corresponding total leaf dry weight to determine the total leaf area.

EXPERIMENTAL RESULTS

I. *The interrelationship between light intensity, net assimilation rate, leaf-area ratio, relative growth rate, and the stage of development of* Helianthus annuus

Before valid comparisons can be made between different experiments it is necessary to determine how far small differences in the stage of development affect the relationship between light intensity and net assimilation rate, leaf-area ratio and relative growth rate. In order to investigate the extent of these variables an experiment was conducted with groups of pots in which sunflower seedlings had emerged 24–5, 22–3, and 14–15 days before the first sample was taken and the screens (0·50 and 0·24 daylight) placed in position.

From Fig. 1*a* it is clear that the linear relationship between net assimilation rate and the logarithm of the light intensity are independent of age (for a fuller discussion, see Blackman and Wilson, 1950). It is equally apparent from Fig. 1*b* that the leaf-area ratio is also linearly related to the logarithm of the light intensity, but in contrast to the trend for net assimilation rate the ratio increases with decreasing intensity. Moreover, there are marked and statistically significant differences between the means for the different emergence dates, thus demonstrating a change in leaf-area ratio as the plants grow older.

The slope of the regression lines measures the sensitivity of the leaf-area ratio to shading, i.e. the plasticity of the plant to changes in light intensity. In order, however, to compare the relative increases of leaf-area ratio brought about by falling light intensity, either between species or within species, this sensitivity must be related to the leaf-area ratio at some standard light intensity. This ratio of regression coefficient (a) to leaf-area ratio (x) at the standard light value will vary inversely with the difference (z) between the logarithm of the light intensity at which the extrapolated regression line passes through the point $Y = 0$ (see Fig. 2*a*) and the logarithm of the standard light value which will be a constant, i.e. the value of the constant will be 2·0 if full daylight for each experiment is taken as 100. The hypothetical value of the light intensity at which the leaf-area ratio becomes zero will be termed the 'leaf-area ratio extinction point' and is a measure of the plasticity of leaf development in response to shade.

The leaf-area ratio extinction points for the three dates of emergence are 3·82, 3·92, and 3·79, from which it follows that within the age range investigated the plasticity of leaf-area ratio in sunflowers is independent of age.

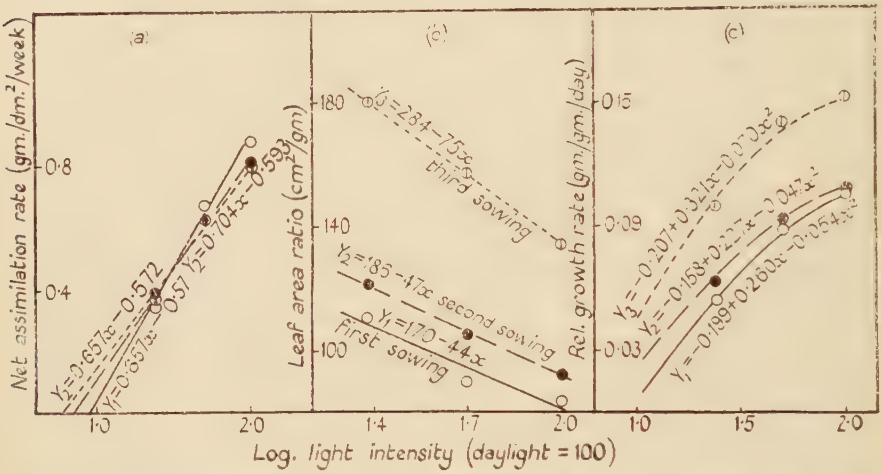

FIG. 1. Expt. 1. *Helianthus annuus.* The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate of plants of different age.

It has already been pointed out in the introduction that relative growth rate is the product of net assimilation rate and leaf-area ratio, and since the net assimilation rate is independent of age (vide Fig. 1*a*) it follows that the differences in relative growth rate at all light intensities will be determined by changes in leaf-area ratio (compare Figs. 1*b* and 1*c*). In this experiment the increases in leaf-area ratio due to the shading treatments do not compensate for decreasing net assimilation rate, and the relative growth rate is therefore depressed at the intensities below full daylight. There is in addition a significant effect of age, since the leaf-area ratios for the youngest plants are the highest; in consequence, the relative growth rates are also higher. It is, however, apparent from Fig. 1*c* that the curvilinear relationships of growth rate against the logarithm of light intensity are similar for each emergence date. Another striking feature of Fig. 1*c* is the extremely good fit of the relative growth-rate values, calculated from the field data, with the curvilinear relationship derived from the two linear regressions.

This relationship of the quadratic form will be a symmetrical curve about the light-intensity axis (see Fig. 2*b*). The relative growth rate will be zero at the 'compensation-point', i.e. the light intensity at which the net assimilation rate is zero. The relative growth rate will also be zero at the light value for the extinction point, for then the leaf-area ratio will theoretically be zero.

The maximum value for the relative growth rate is—on the logarithmic scale—midway between the compensation point and the extinction point.

From the foregoing results it is evident that the effects of shading on the growth of any species can be analysed with a new precision once the relationships with light intensity of both net assimilation rate and leaf-area ratio have been established.

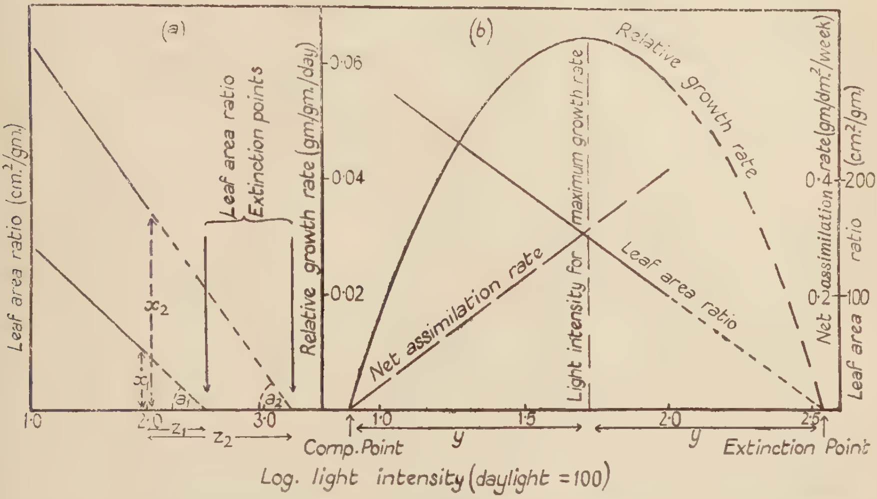

FIG. 2. (*a*). The relationship between the extinction-point value and the ratio of regression coefficient (*a*) of leaf-area ratio on light intensity to the leaf-area ratio in full daylight (*x*); see text. (*b*) Schema showing the quadratic relationship between relative growth rate and the logarithm of light intensity derived from the product of the linear regressions of net assimilation rate and leaf-area ratio on the logarithm of light intensity.

II. *The adaptation of* Helianthus annuus *to shading in relation to net assimilation rate, leaf-area ratio, and relative growth rate*

In each of the present experiments, prior to the screens being placed in position, all the plants received full daylight and in consequence, during the initial phase of each experiment, the effects of shading will have included adjustment to the different light levels, i.e. the morphological and physiological changes concerned with adaptation. Information as to the length of this period of adjustment is forthcoming from some six sunflower experiments, in which sampling was carried out on three or more occasions.

The results from three comparable experiments (expts. 2, 3, and 4) are shown in Figs. 3, 4, and 5. In each experiment and for the two consecutive experimental periods there is a close linear relationship between the logarithm of light intensity and both net assimilation rate and leaf-area ratio. It has already been demonstrated that within limits the relationship between light intensity and net assimilation is largely independent of stage of development (Blackman and Wilson, 1950), but the results of expt. 1 have also shown that, while the leaf-area ratio increases directly with the logarithm of decreasing light intensity, the ratio decreases with age. Therefore the interpretation of differences in the leaf-area ratios for successive periods in one experiment or differences between experiments must allow for this ontogenetic drift.

The results for these three experiments (Figs. 3–5) indicate that the plants were not completely adjusted to the light environments by the end of the first period, i.e. 8 to 10 days. At the instant of commencing the experiment the leaf-area ratios are identical at all light intensities and the relationship with light intensity is represented by a line parallel to the light axis. If samples are taken before the plants are fully adjusted to each light level, the leaf-area ratio regression will be intermediate between zero and the figure when the full potential for modification is realized. Expressed in another way, the value of the extinction point will change from infinity to a minimum value when adaptation to the light level is complete. These considerations, however, assume that during the period of modification the leaf-area ratio in full daylight is a constant, whereas in practice the ratio will tend to fall, and this in turn will shift the minimum value of the extinction point.

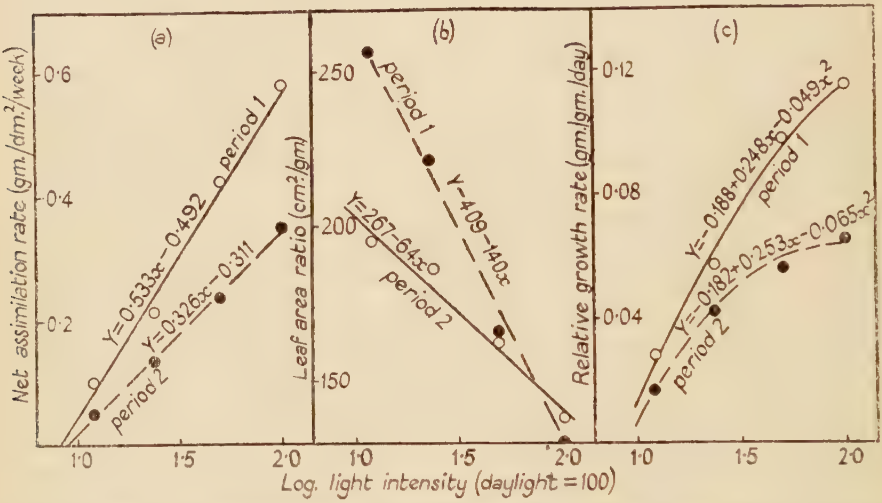

FIG. 3. Expt. 2. *Helianthus annuus*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over consecutive periods (5–14–23/9/47).

From Figs. 3–5 it is evident that there is a general trend for the slope of the regression line in the second period to be greater than in the first. In spite, however, of the incomplete adjustment the logarithmic relationship is maintained throughout each experiment.

This process of adjustment has masked the tendency for the leaf-area ratio to decrease with age since at the lower light intensities the ratios invariably increase between the first and second periods. In consequence, the mean leaf-area ratio in each experiment is significantly greater in the second period.

Turning next to a consideration of the effects of light intensity on the relative growth rate, there is for all three experiments and occasions a good agreement between the observed values and the values calculated from the quadratic equations obtained by multiplying together the appropriate regressions of net assimilation rate and leaf-area ratio. It should in addition be noted that in each experiment the shape of the curve is different for the two periods. For the first period the curves suggest that the maximum growth

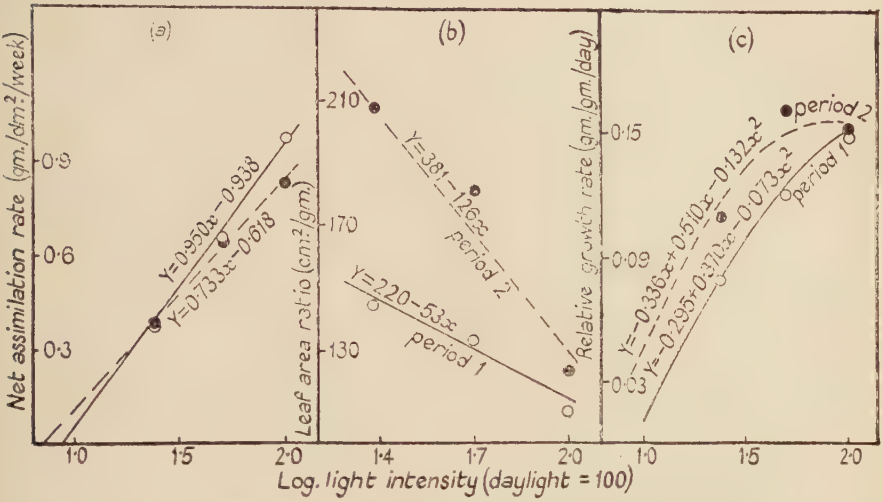

FIG. 4. Expt. 3. *Helianthus annuus.* The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over consecutive periods (14–24–8/7/48).

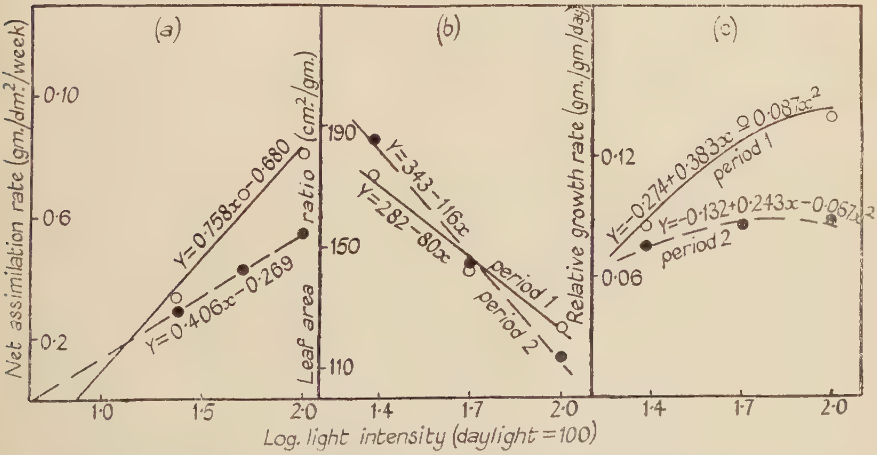

FIG. 5. Expt. 4. *Helianthus annuus.* The effect of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over consecutive periods (19–27/8–4/9/47).

rate would have been achieved at light intensities in excess of full daylight, while for the second period the maximum has shifted to a point equal to or below full daylight. This shift is another characteristic of the process of adjustment to shading, for if the regressions for net assimilation rate between periods are somewhat similar, as in expt. 3, then changes in the slope of the regression for leaf-area ratio will be reflected in changes of relative growth rate. From Fig. 4 it is apparent that the higher regression coefficient for leaf-area ratio in the second period has resulted in a much flatter curve for relative growth rate. In other words, the light intensity at which the maximum growth rate will take place cannot be assessed until the leaf-area ratios have become fully adjusted to the light conditions.

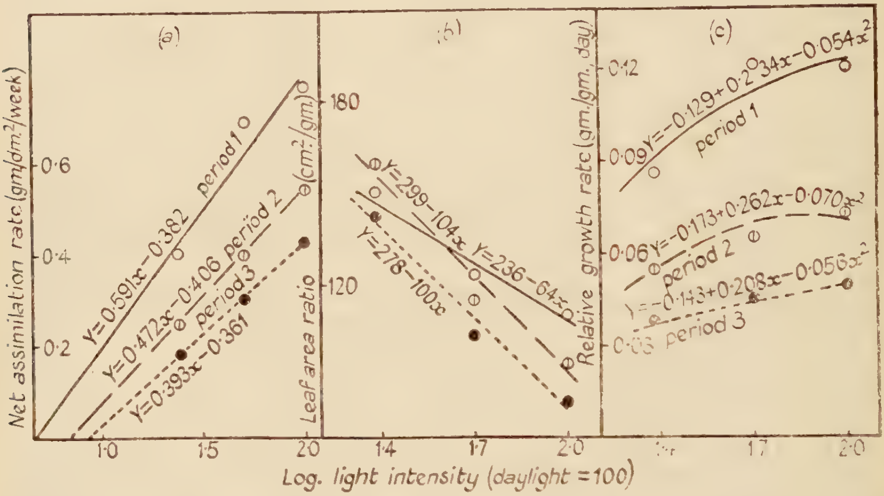

FIG. 6. Expt. 5. *Helianthus annuus.* The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over consecutive periods (15–24/5–4/6/48).

Whereas in expts. 2 and 3 the conditions of growth were normal, in expt. 4 there was some indication of nutrient deficiency and the growth rates fell sharply in the second period. Similarly in expts. 5 and 6 the conditions were abnormal since during the experimental period the pots became waterlogged due to the silting up of the drainage-holes as a result of heavy rain. The plants looked unhealthy and grew somewhat slowly, and in expt. 6 the data for the *third* experimental period have been discarded, since by this time some necrosis of the leaves had occurred.

The results for these two experiments are shown in Figs. 6 and 7, and it is apparent that in spite of the conditions the linear relationships between net assimilation rate, leaf-area ratio, and the logarithm of the light intensity still hold for the first two periods in expt. 6 and all periods of expt. 5. In both experiments there is again evidence that at the end of the first period full adaptation in terms of leaf-area ratio had not taken place. This progressive adjustment with time is also seen in the changing shape of the curves relating relative growth rate and the logarithm of the light intensity for the different periods (Figs. 6*c* and 7*c*). In expt. 5 the decrease in the rate of growth over successive periods is determined by changes in net assimilation rate rather than by changes in leaf-area ratio.

It has already been emphasized in the introduction that the estimation of either the mean net assimilation rate or the mean leaf-area ratio between two sampling occasions is liable to error if the changes in leaf area are not linearly related to changes in total plant weight. The errors introduced when correlating light intensity and net assimilation rate have been previously discussed (Blackman and Wilson, 1950), but so far no consideration has been given to leaf-area ratio.

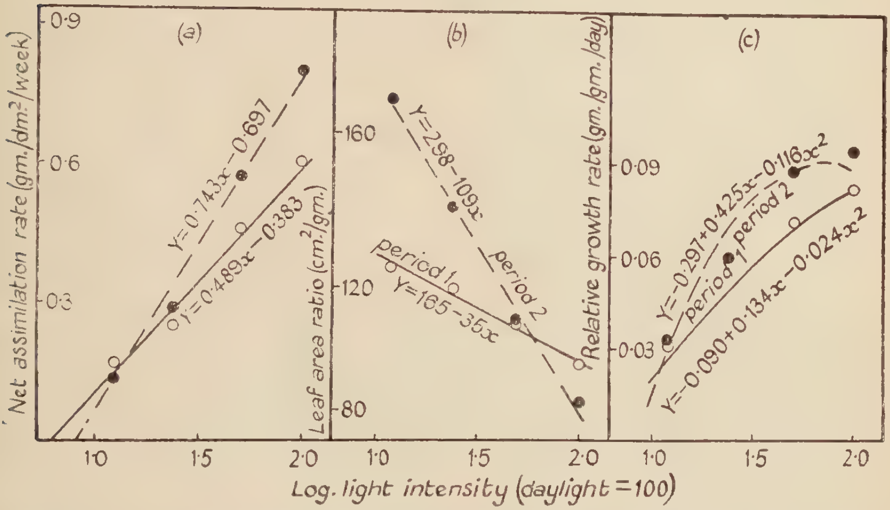

FIG. 7. Expt. 6. *Helianthus annuus*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over consecutive periods (21/4–5–14/5/48).

In expts. 2–6, for each light intensity the changes in plant weight relative to leaf area are approximately linear (see Figs. 2–6, Blackman and Wilson, 1950), but in expt. 7 this is not so (Fig. 8*a*). On general grounds, it is evident that the shorter the time interval between sampling dates the less will be the error introduced, and some estimate of the error can be obtained from expt. 7 by calculating the mean leaf-area ratios between the first and third samples and comparing them with the mean figures derived from the first and second samples and the second and third samples respectively. The differences obtained are seen in Fig. 8*b*. It is apparent that with a non-linearity of the leaf-area to plant-weight relationship, changes in the leaf-area ratios are underestimated, but nevertheless the error is proportionate and the ratio is still logarithmically related to light intensity.

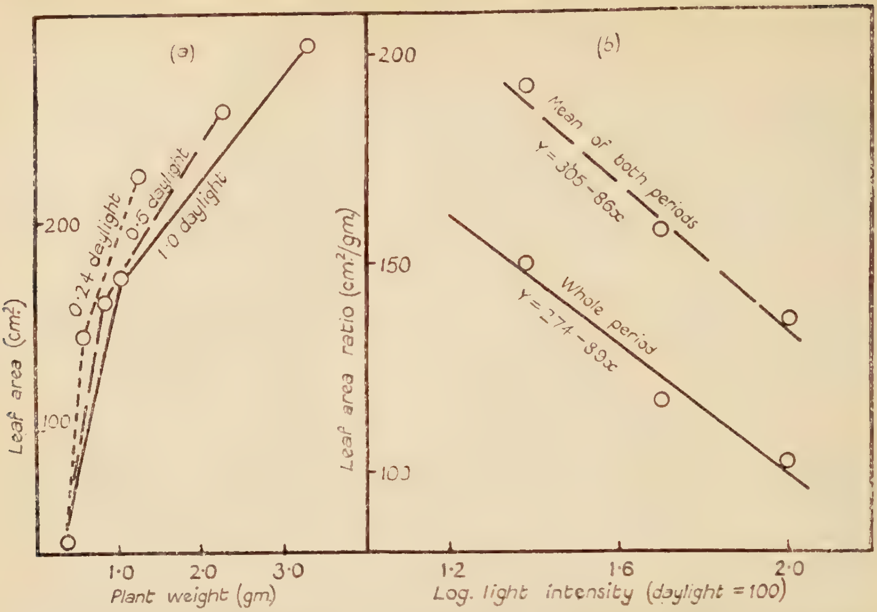

FIG. 8. Expt. 7. *Helianthus annuus*. (*a*) The effects of varying light intensity on the relative changes in leaf area and plant weight at successive sampling occasions (11–19–27/7/47). (*b*) The errors introduced into the linear relationship between leaf-area ratio and the logarithm of the light intensity when changes in plant weight are not proportional to changes in leaf area. Divergence between mean regression of first and second period and regression based on data of initial and final samples.

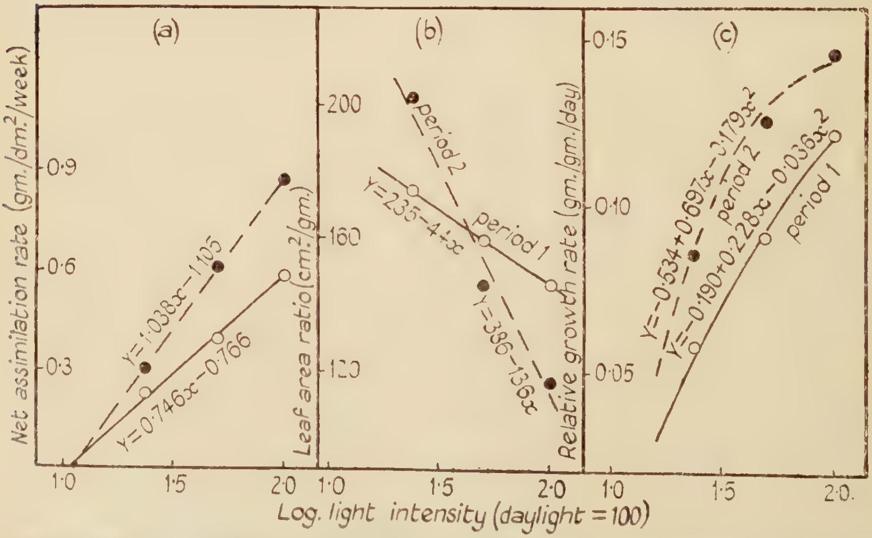

FIG. 9. Expt. 7. *Helianthus annuus*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over successive experimental periods (11–19–27/7/47).

Bearing these errors in mind, it can nevertheless be concluded from Fig. 9 that the plants were not fully adjusted to shading at the end of the first experimental period. It can also be concluded that in spite of the changes in leaf area not being proportional to changes in plant weight, the quadratic equations obtained from the regressions of light intensity of net assimilation rate and leaf-area ratio still fit the observed data for relating growth rate.

TABLE I

The Change with Sampling Occasion in the Value of the Extinction-Point Intensity for Helianthus annuus

	Extinction point Log. light intensity				Relative growth rate at 0·5 daylight—g./g./day		
Expt.	Per. i.	Per. ii.	Per. iii.	Last sample.	Per. i.	Per. ii.	Per. iii.
2	4·17	2·95	—	2·79	0·10	0·06	—
3	4·11	3·02	—	2·55	0·12	0·16	—
4	3·52	2·96	—	3·00	0·14	0·09	—
5	3·70	2·87	2·78	2·68	0·12	0·07	0·05
6	4·17	2·95	—	2·79	0·07	0·09	—
7	5·34	2·84	—	2·79	0·09	0·13	—

The effects of increasing the period of shading on the adaptation of the leaf-area ratio can be compared between the several experiments by examining the change in the value of the leaf-area extinction point, since it has already been pointed out (see p. 380) that this will decrease at a slow rate once full modification has taken place. Table I gives the values of the mean extinction points over successive periods together with the extinction point derived from the leaf-area ratio regression for the last sampling occasion, i.e. when the plants had been shaded for 14 to 27 days. It is apparent that most of the adaptation has already taken place by the end of the first experimental period and that the extinction-point values for the last sampling occasion are representative of full modifications. It is emphasized that adaptation will proceed fastest where the growth rate is high, but at the same time the ontogenetic drift will also be emphasized. Thus the difference between the values for the second period and the last sampling occasion are greatest in expt. 3, which also had the largest relative growth rates. Again, in expt. 4 nutrient deficiency may have been a factor in slowing down the rate of adaptation, for apart from this experiment there is good agreement between the values for the last sampling occasion.

III. *The interaction between light intensity and mineral nutrient supply on the net assimilation rate, leaf-area ratio, and relative growth rate of* Helianthus annuus

Out of the eleven field experiments conducted at Slough, in only two did the combined addition of nitrogen, phosphorus, and potassium have appreciable effects on growth and development. These effects of nutrient supply in relation to light intensity have been analysed for each experiment into the effects on net assimilation rate, leaf-area ratio, and relative growth rate.

The results for expt. 12 are set out graphically in Fig. 10. Considering first net assimilation rate, there is an interaction between light intensity and the level of nutrient supply since only at the higher light intensities is there an increase in net assimilation rate (Fig. 10*a*). On the other hand, for leaf-area ratio there is no interaction between light intensity and nutrient level since at all light intensities there has been a proportionate increase in the ratio. The relative growth-rate curves derived from the pairs of regressions for low and high nutrient supply are shown in Fig. 10*c*, and once more there is good agreement with the observed data.

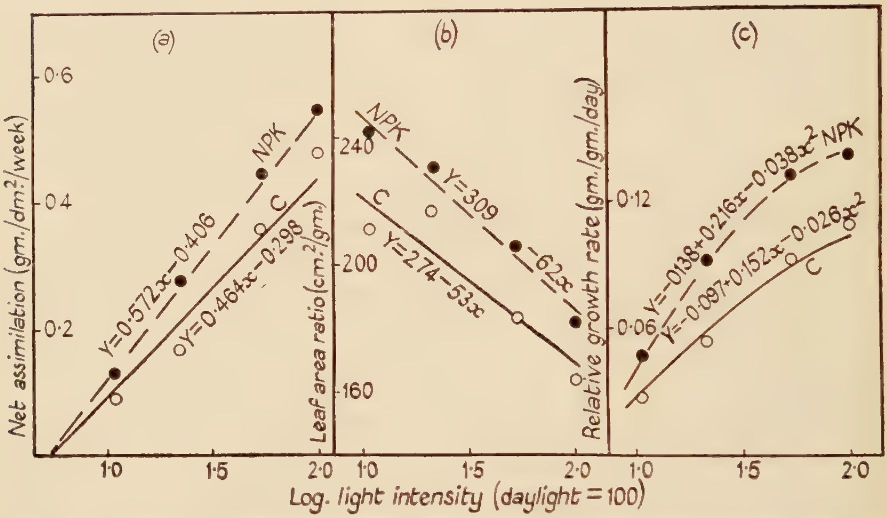

FIG. 10. Expt. 12. *Helianthus annuus.* The effects of mineral nutrient level and light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate (31/7–8/8/42).

In expt. 11 there is again for net assimilation rate a significant effect of nutrient supply with the greater increases at the higher light intensities. In contrast to expt. 12 the higher level of nutrient supply has not increased the leaf-area ratio (Fig. 11*b*). In consequence, the changes in relative growth rate with light intensity reflect the differences between the two regressions for net assimilation rate. As in expt. 12, the increase in relative growth rate due to additional nutrients is maximal in full daylight and negligible at the lowest light intensity—0·11 daylight.

IV. *The effects of varying light intensity on the net assimilation rate, leaf-area ratio, and relative growth rate of different species*

(*a*) *Fagopyrum esculentum.* Two pot experiments were conducted with buckwheat in which there were three sampling occasions. Since the interval between full daylight and 0·5 daylight is large, an additional light intensity of 0·67 daylight was included in expt. 20. Fig. 12 *a* and *b* shows that between the two experimental periods the regressions for net assimilation rate are

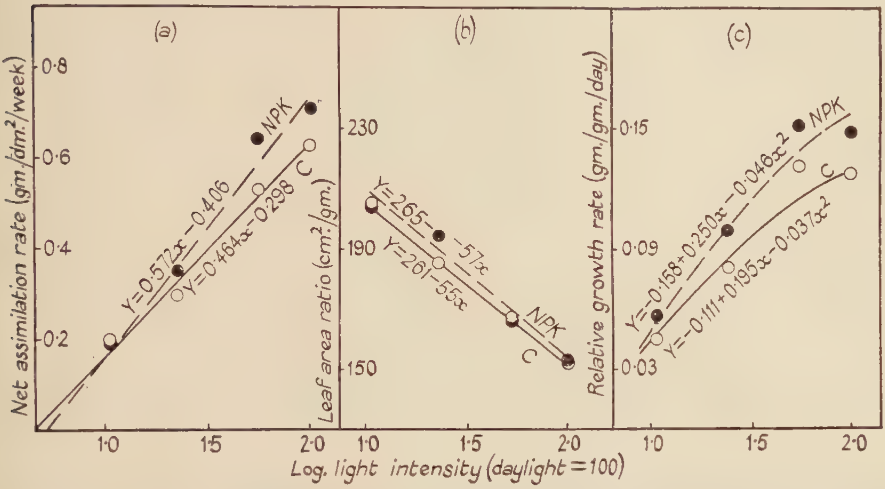

FIG. 11. Expt. 11. *Helianthus annuus.* The effects of mineral nutrient level and light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate (25/6–3/7/42).

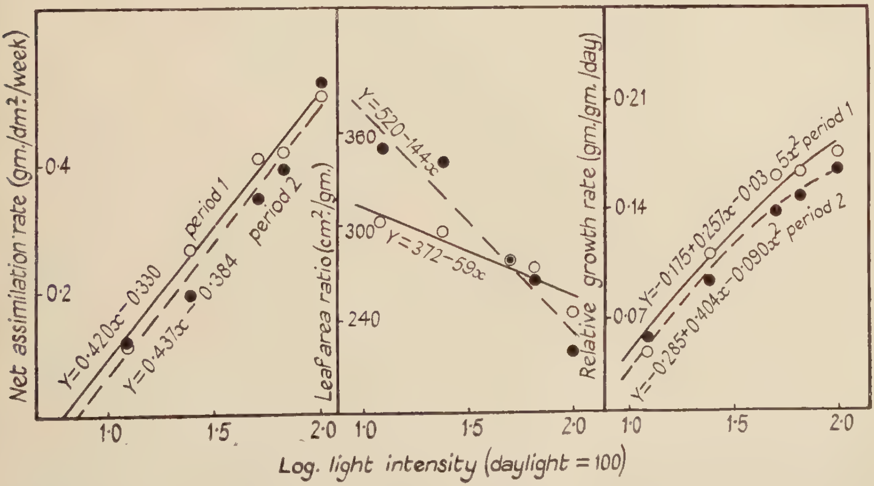

FIG. 12. Expt. 20. *Fagopyrum esculentum.* The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over successive periods (21–30/8–6/9/48).

very similar, but that the slope of the regression for leaf-area ratio is higher in the second period. As in the case of the sunflower, this change is taken as evidence that by the end of the first period the plants were not fully adjusted to the various degrees of shading. Again it is apparent from Fig. 12*c*

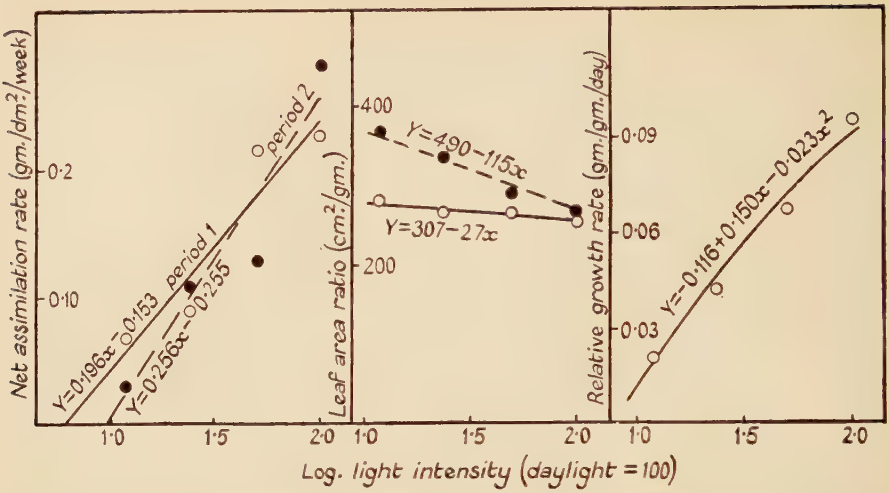

FIG. 13. Expt. 21. *Fagopyrum esculentum*. The effects of varying light intensity over successive periods on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate for the periods combined.

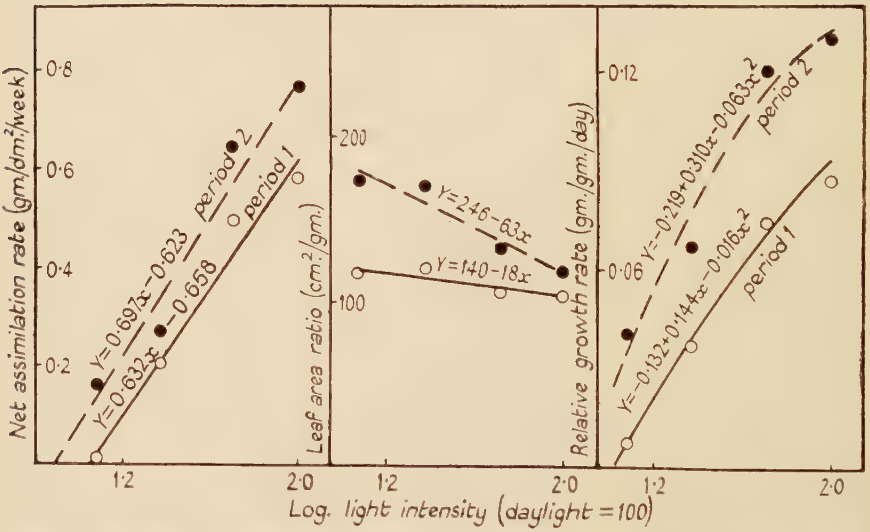

FIG. 14. Expt. 22. *Trifolium subterraneum*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over successive periods (31/7–9–18/8/47).

that there is a reasonably good agreement between the observed and calculated values of relative growth rate.

Clearly in expt. 21 (Fig. 13) the departures of the observed net assimilation rates from the regressions are large, and because of this lack of uniformity only the curve for the mean relative growth rate over the whole experimental period has been calculated from the regressions of mean net assimilation rate and leaf-area ratio. This curve fits the observations well and suggests that the errors for assimilation were due to a combination of the small changes in weight during the experiment and insufficient replication.

(*b*) *Trifolium subterraneum.* The results for the single experiment on *T. subterraneum* are given in Fig. 14. In the case of net assimilation rate, higher values were obtained in the second period (Fig. 14*a*), and this is equally true for leaf-area ratio. In fact for the first period the smallness of the regression coefficient suggests that in subterranean clover the rate of adjustment to shade conditions is slow. Moreover, the shapes of the curves for relative growth rate between half and full daylight indicate that this species is intolerant of even light shade (Fig. 14*c*).

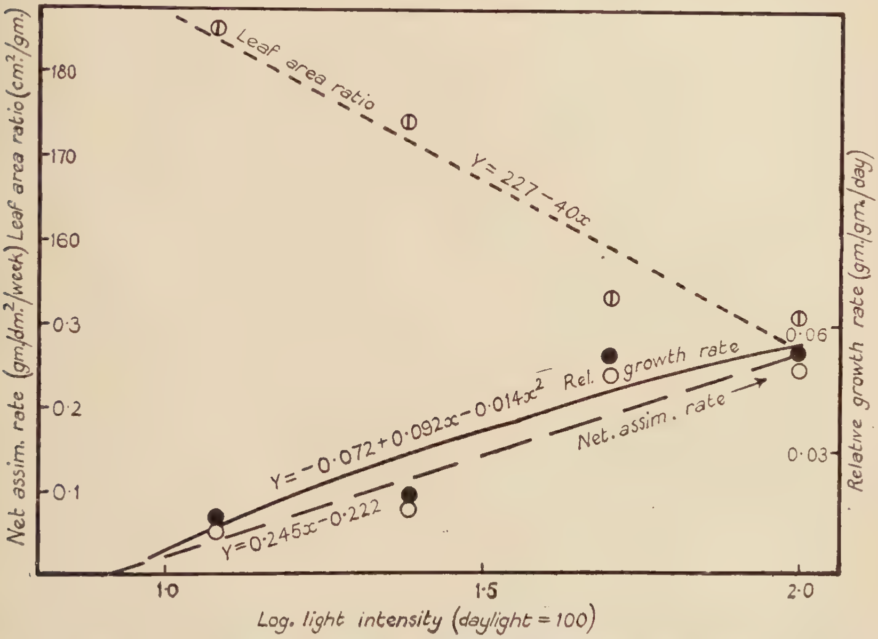

FIG. 15. Expt. 23. *Tropaeolum majus.* The effects of varying light intensity on the net assimilation rate, the leaf-area ratio, and the relative growth rate (19/9–2/10/47).

(*c*) *Tropaeolum majus.* This single experiment was carried out late in the autumn when the changes in plant weight were small and in consequence the experimental errors high. Nevertheless, the general trends are common to this and the previous species (Fig. 15). The regression for leaf-area ratio is the closest fit to the data, and the variations in relative growth rate are thus largely related to the discrepancies between the observed data and calculated regression for net assimilation rate.

(*d*) *Lycopersicum esculentum.* The results obtained for the tomato fully support the conclusions reached for the sunflower. From Fig. 16 it is evident that both for net assimilation rate and leaf-area ratio the agreement between the data and the calculated linear regressions is good. In consequence, the

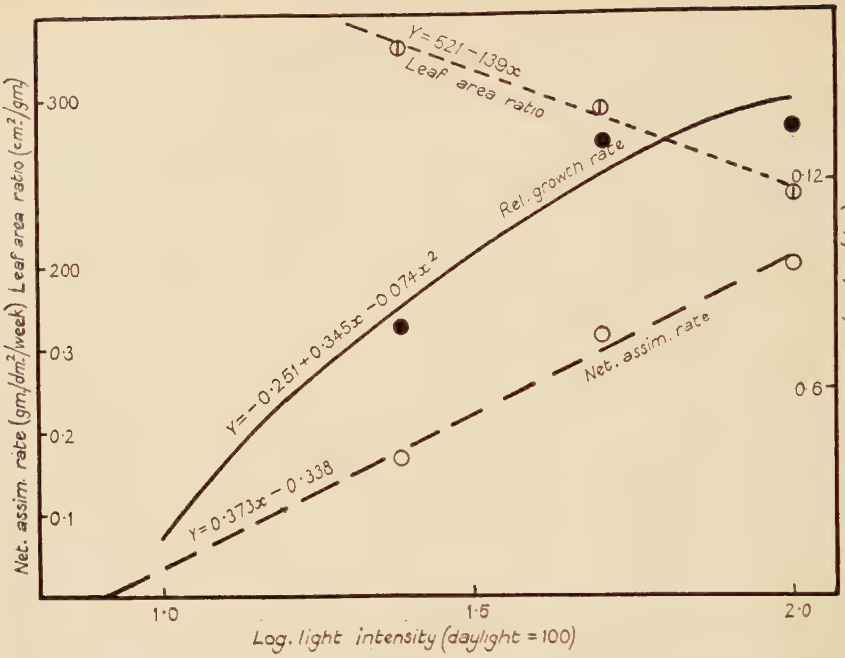

FIG. 16. Expt. 24. *Lycopersicum esculentum*. The effects of varying light intensity on the net assimilation rate, the leaf-area ratio, and the relative growth rate (3–11/9/48).

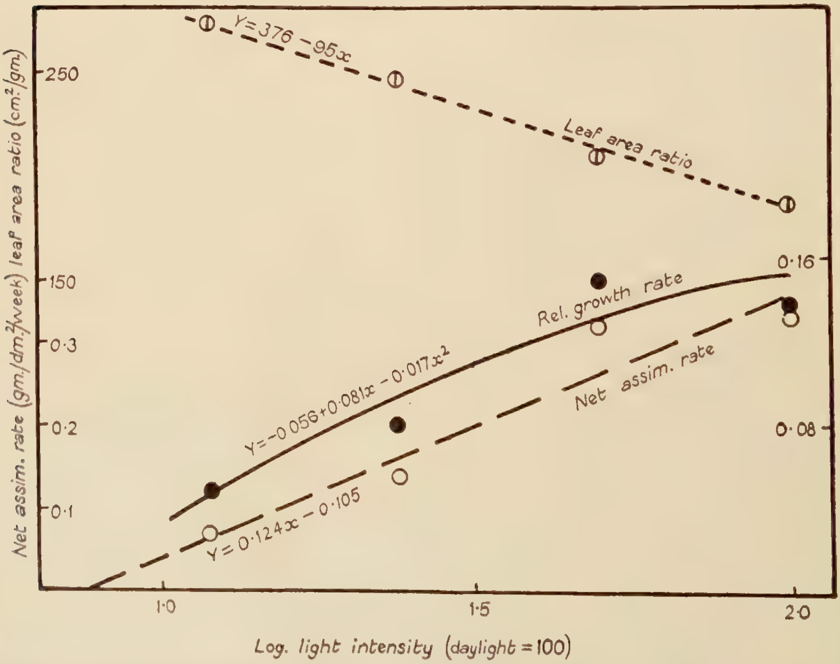

FIG. 17. Expt. 25. *Solanum dulcamara*. The effects of varying light intensity on the net assimilation rate, the leaf-area ratio, and the relative growth rate (24/9–12/10/47).

theoretical relationship between light intensity and relative growth rate based on the pair of regressions is in line with the rates estimated from the changes in plant weight.

(*e*) *Solanum dulcamara.* The experiment with this species was undertaken in the autumn and therefore the rate of growth was slow. Under these conditions the net assimilation rates and leaf-area ratios are linearly related to the logarithm of the light intensity and once more the calculated relationship for relative growth rate and light intensity fits the observed data reasonably well (Fig. 17).

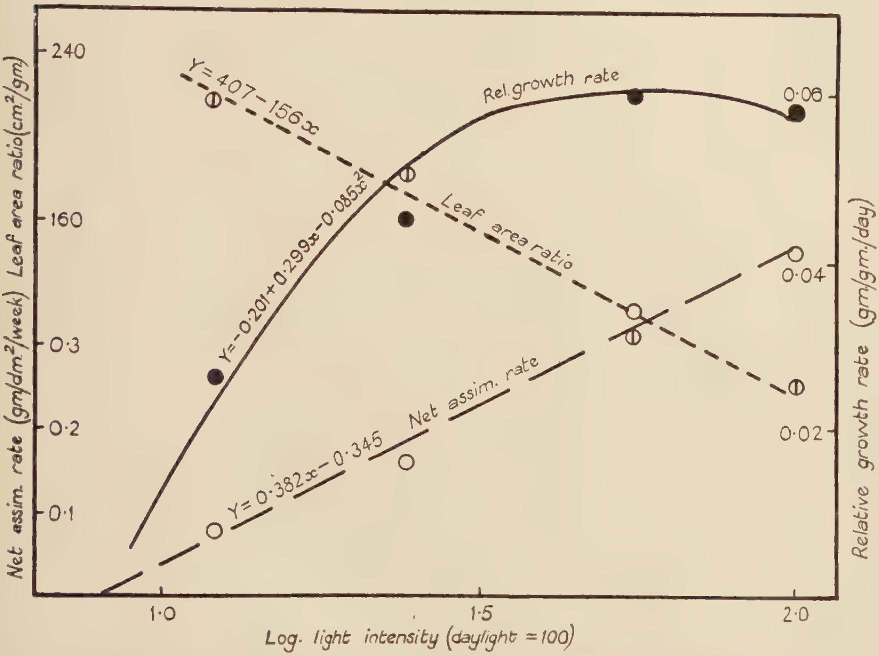

FIG. 18. Expt. 26. *Geum urbanum.* The effects of varying light intensity on the net assimilation rate, the leaf-area ratio, and the relative growth rate (24/8–14/9/48).

(*f*) *Geum urbanum.* Although the conditions were relatively favourable to growth, this species grew slowly, and in order to reduce the experimental errors the period between sampling occasions was extended to 3 weeks. From Fig. 18 it is apparent that the regressions for net assimilation rate and leaf-area ratio, together with the quadratic equation for relative growth rate, all fit the data with precision.

V. *The comparative effects of varying light intensity on the net assimilation rate, leaf-rate ratio, and relative growth rate of* Fagopyrum esculentum *and other species*

(*a*) *Fagopyrum esculentum* and *Helianthus annuus.* In comparisons between species maximum precision can only be achieved when simultaneous observations are made under the same set of environmental conditions. Accordingly a number of experiments have been carried out in which *F. esculentum* has been grown in the same experiment with a number of other plants. Fig. 19 gives the relevant data for expt. 27, in which buckwheat and sunflower were compared during a period of rapid growth.

From Fig. 19*c* it is evident that the effects of shading on the relative growth rate of both species are very similar, although *F. esculentum* grows somewhat faster. Inspection of Fig. 19*a* and *b* demonstrate that this higher rate of growth is due to the greater leaf-area ratio of *F. esculentum*, which more than offsets the slightly higher rate of assimilation of the sunflower.

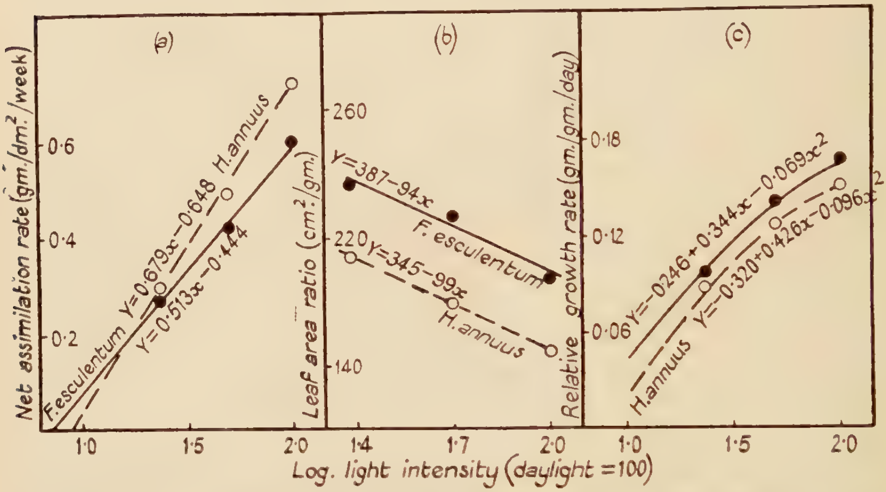

FIG. 19. Expt. 27. *Fagopyrum esculentum* and *Helianthus anmuus*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate (10–20/8/48).

(*b*) *Fagopyrum esculentum* and *Vicia faba*. A comparison between *F. esculentum* and *V. faba* was not undertaken until the end of September, and it would be expected that the cold nights and slight ground frosts should have been more inimical to buckwheat than to the winter variety of field bean. Nevertheless, Fig. 20*c* demonstrates that irrespective of the light intensity *F. esculentum* has a much higher relative growth rate. This major difference can be ascribed to the variations in the leaf-area ratio of the two species together with the greater adjustment of *F. esculentum* to falling light intensity (Fig. 20*b*) rather than to the somewhat larger net assimilation rate of *V. faba*, which is more than counterbalanced by the higher leaf-area ratios of *F. esculentum*.

(*c*) *Fagopyrum esculentum*, *Helianthus annuus*, and *Hordeum vulgare*. Expt. 29 consisted of a comparison of *F. esculentum* with *H. vulgare*, but relevant data are available for *H. annuus* which was part of another experiment sampled on the same occasions. However, since the soil in the sunflower pots

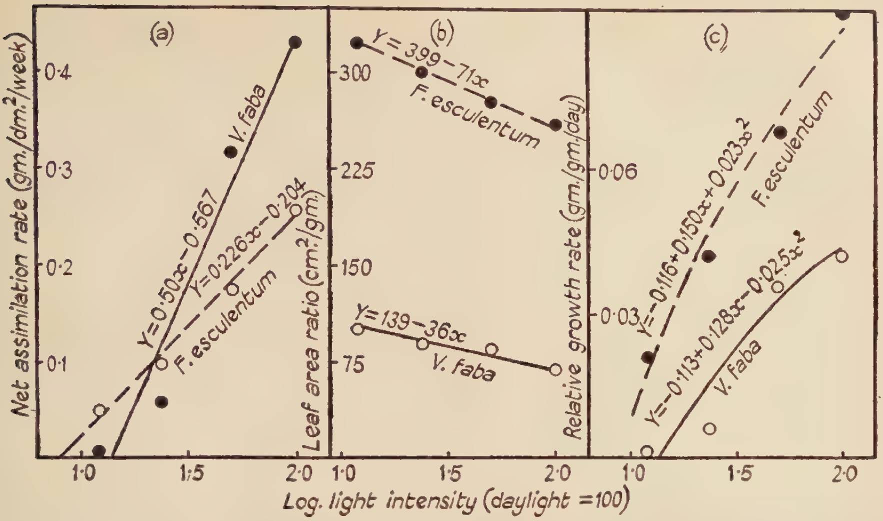

FIG. 20. Expt. 28. *Fagopyrum esculentum* and *Vicia faba*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate (25/9–13/10/47).

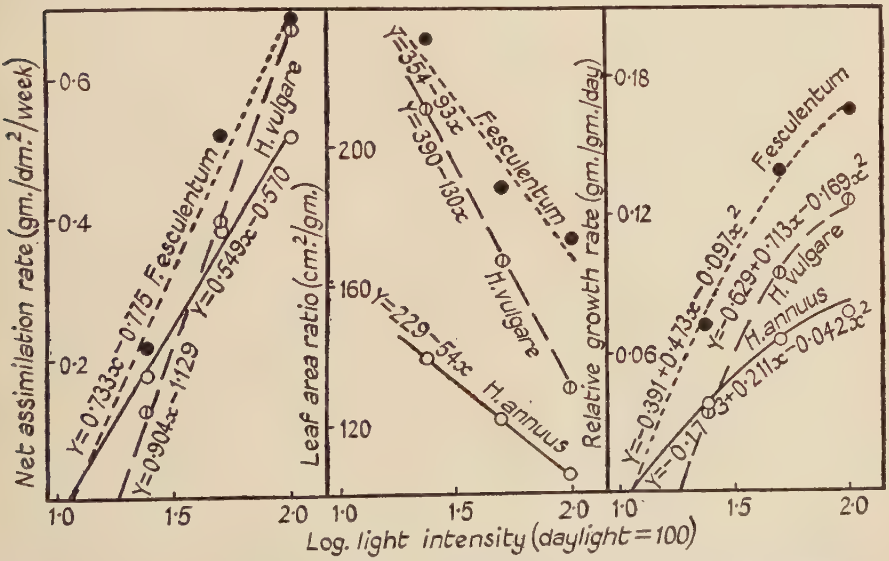

FIG. 21. Expt. 29. *Fagopyrum esculentum*, *Hordeum vulgare*, and *Helianthus annuus*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate (12–21/7/47).

was different, some source of error is thereby introduced, but is unlikely to be large.

Considering first the results for net assimilation rate (Fig. 21), in full daylight the values for *F. esculentum* and *H. vulgare* are greater than the figure for *H. annuus*. On the other hand, since the slope of the regression is steepest in the case of *H. vulgare*, this species has the highest compensation point and the least effective assimilation at the lowest light intensities. Turning next to the leaf-area ratio, decreasing light intensity has caused a similar effect for all three species, but since in full daylight *F. esculentum* has the highest and *H. annuus* the lowest ratio, the mean values over all light intensities are largely proportionate to those in full daylight. These differences between species are reflected in the relative growth-rate curves (Fig. 21*c*). Because of the sharp falling-away of net assimilation rate with decreasing light intensity, shading causes a commensurate fall in the growth rate of *H. vulgare*. Since for the other two species the regression coefficients for net assimilation rate are smaller, at the lower intensities both species grow faster than barley. As *F. esculentum* has both the highest assimilation rates and the largest leaf-area ratios, it also has the highest growth rates.

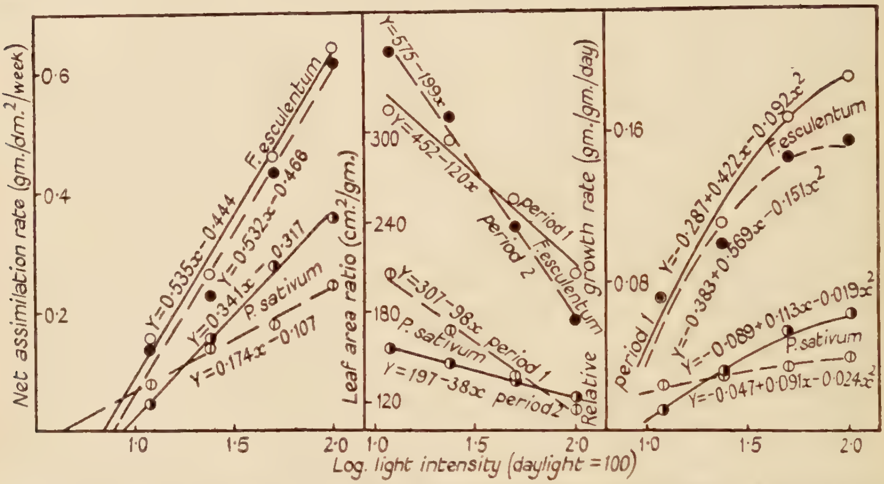

FIG. 22. Expt. 30. *Fagopyrum esculentum* and *Pisum sativum*. The effects of varying light intensity on (*a*) the net assimilation rate, (*b*) the leaf-area ratio, and (*c*) the relative growth rate over successive periods (18–29/6–7/7/48).

(*d*) *Fagopyrum esculentum* and *Pisum sativum*. The comparison between buckwheat and *P. sativum* was extended over two consecutive experimental periods, but heavy rain eventually led in the second period to the waterlogging of the pots. These conditions differentially affected the two species. As far as visual observation went, and this is supported by the experimental data, the conditions were not inimical to the buckwheat but adverse to the pea. For example, while with *F. esculentum* the regressions of net assimilation rate on light intensity are very similar for the two periods, the slope of the regression and the mean value for *P. sativum* in the second period is less than in the first (Fig. 22*a*). For leaf-area ratio, the trends for both species are the same in that the regression coefficients for the second period are greater than in the first. Since, however, over both periods *F. esculentum* has higher net assimilation rates and greater leaf-area ratios, the relative growth rates at each light intensity far exceed those of *P. sativum* (Fig. 22*c*).

DISCUSSION

From the results that have been presented the conclusion can be reached that the general effects of shading on growth rate in the early vegetative phase can be analysed with a new precision. The basis of this analysis rests on the linearity of the relationships between the logarithm of the light intensity and both net assimilation and leaf-area ratio. The consistency of this relationship between daylight and 0·1 daylight in the case of net assimilation rate has been discussed previously (Blackman and Wilson, 1950), but there remains for consideration the closeness of fit of the data for leaf-area ratio.

In each of the twenty pot experiments undertaken at Oxford, an analysis of variance was carried out to test whether departures from the linear regressions of leaf-area ratio on the logarithm of the light intensity were appreciable, and only in a small proportion of the experiments (expts. 3, 5, 8, 20, and 22) did the departures reach a level of significance. Except, however, in expt. 8, the variance for the departure only represented a small fraction (2·5 to 6·1 per cent.) of the total variance for the regression. In the remaining experiments there was little evidence of non-linearity, and if such departures were the normal trend, then they should have been detected, since in expts. 1, 4, 6, 7, 24, 25, 27, 29, and 30 the errors were low, i.e. the coefficient of variation was from 1·4 to 7·3 per cent.

Thus on the basis of these findings it can be concluded that from the viewpoint of the analysis of the effects of shading, there is no consistent departure from a linear relationship either between or within species. Just as it was emphasized for net assimilation rate in the previous paper (Blackman and Wilson, 1950), it is again stressed that under some conditions small departures from linearity may be found. Indeed, considering that the leaf-area ratio is determined by a combination of many factors, it would be surprising if the linear relationship was exact. On the other hand, the relationships established for leaf-area ratio and net assimilation rate are sufficiently precise for the effects of shading on the relative growth rate of plants in the open to be predicted with considerable accuracy.

For leaf-area ratio, there is some indication that departures from linearity may result from unfavourable conditions. For example, expt. 8 was carried out at the end of September at relatively low temperatures, while in expt. 5 the curvilinearity is most evident in the third period, when the plants were waterlogged.

For any precise analysis of any interaction between temperature and light intensity on the leaf-area ratio, a range of chambers or greenhouses with automatic controls is clearly demanded, but since these were not available it was thought that some information could be obtained by comparing the growth of plants in a greenhouse with others grown in the open. The experimental details have been given in the previous paper (Blackman and Wilson, 1950); briefly, plants with or without additional shading were grown in a normal greenhouse simultaneously with plants grown in the open under a series of screens and the net assimilation rates, leaf-area ratios, and relative growth rates found in the usual way. The relative light intensities in the greenhouse were determined and the net assimilation rates and leaf-area ratios of the outdoor plants at levels corresponding to the greenhouse intensities were estimated from the regression equations, calculated from the data of the outdoor plants. It is obvious that any observed differences can only be described as a 'greenhouse effect', and although the assumption is probably valid that the main effect was the higher temperature—5°–8° C.—other factors may also operate.

TABLE II

The Relative Growth of Plants grown in a Greenhouse and in the Open at Equivalent Light Intensities

Experiment and species.	Net assimilation rate g./100 $cm.^2$/week		Leaf-area ratio $cm.^2$/g.		Relative growth rate g./g./day		Mean daily temperatures in open.	
	open	green-house.	open	green-house.	open	green-house.	Max. ° C.	Min. ° C.
Fagopyrum esculentum								
Expt. 20. 0·7 daylight								
Period 1 . .	0·445	0·424	263	266	0·167	0·160	20·1	11·3
Period 2 . .	0·422	0·362	254	251	0·153	0·127	20·4	11·5
Expt. 30. 0·7 daylight								
Period 2 . .	0·516	0·461	208	261	0·153	0·173	17·5	9·8
Helianthus annuus								
Expt. 3. 0·7 daylight	0·814	0·960	122	108	0·153	0·148	20·0	12·2
Expt. 8. 0·7 daylight	0·334	0·433	171	195	0·082	0·121	19·0	10·0
0·47 daylight	0·266	0·269	179	204	0·068	0·078		
0·35 daylight	0·212	0·237	182	213	0·055	0·074		
Lycopersicum esculentum								
Expt. 24. 0·7 daylight	0·350	0·513	265	271	0·133	0·199	20·2	12·2

The results of the five experiments are summarized in Table II. Considering first the net assimilation rate, the indications are that under the greenhouse conditions the rate is higher for *H. annuus* and *L. esculentum* but lower for *F. esculentum*. In expts. 20, 3, and 24 the leaf-area ratios within and without the greenhouse are of the same order, while in expts. 30 and 8 the ratio is less in the open. The temperature data indicate that the differences in ratio are accentuated at lower temperatures. It is also of interest to record that the departure from linearity between leaf-area ratio and the logarithm of the light intensity found for the outdoor plants is absent in the greenhouse plants. From the viewpoint of relative growth rate an appreciable divergence between the indoor and outdoor plants is only apparent in expts. 8 and 24.

Because of the precision with which the linear regressions fit both the data for net assimilation rate and leaf-area ratio in the majority of experiments, it would be expected that the light–growth rate curves determined from the pairs of regression equations should fit the observed values closely. This expectation has been fully realized; indeed, in expts. 1, 2, 12, 27, 29, and 30 (Figs. 1, 3, 10, 19, 21, 22) the agreement for biological data is most striking. Only for *T. majus* (Fig. 15*c*) can the fit be described as indifferent.

It has already been emphasized that although the same general interrelationship between net assimilation rate, leaf-area ratio, and relative growth rate and shading are independent of the length of the experimental period, there is some modification as the length is increased, for the plants take some time before full adaptation to the different light levels has taken place. The major change over consecutive experimental periods relates to the increasing slope of the regression for leaf-area ratio (see p. 385). It therefore follows that the most accurate comparison of the effects of reduced light intensity on the relative growth rate of different species will be attained when the physiological and morphological changes induced by the light level are complete.

In expts. 27 and 29, where direct comparisons have been made between *H. annuus* and *F. esculentum*, it has been shown that the higher growth rates of buckwheat at all light intensities can be attributed to the greater leaf-area ratios rather than differences in net assimilation rate (pp. 392–4). Both these experiments were, however, of relatively short duration, and although the growth rates, particularly for *F. esculentum*, were high, the question arises whether full leaf adaptation had already taken place at the end of the experiments. Some estimate of this can be obtained from a consideration of the values of the leaf-area ratio extinction points derived from the regressions of the leaf-area ratios at the final sampling occasion. For *H. annuus* the values on a logarithmic scale of light intensity are 2·78 and 2·92, and for *F. esculentum* 3·17 and 2·92. These figures are in the same ranges as those derived from the experiments of longer duration when full adaptation is likely to have taken place. Thus in the *H. annuus* experiments (expts. 2–6) the values obtained from the leaf-area ratio regressions at the third or fourth sampling occasion ranged from 2·55 to 3·00 (Table I), while for *F. esculentum* (expts. 20 and 30) the limits are 2·91 and 3·21.

It has already been pointed out (see Fig. 2) that the relative growth rate will be zero at both the compensation point and extinction point and that the maximal growth rate will occur at the light intensity which is midway on a logarithmic scale between the extinction and compensation points. The position of the extinction point will be determined both by the leaf-area ratio in full daylight and the slope of the regression line linking leaf-area ratio with falling light intensity. Thus, since for all the species so far investigated the compensation points, admittedly determined by extrapolation, lie within a narrow range, the intensities at which individual species will attain a maximal relative growth rate will be largely dependent on the value of the extinction point. The shape of the growth-rate curve induced by falling light intensity will be governed by the values of net assimilation rate and leaf-area ratio, but the optimal light level for a maximum growth rate will be independent of the magnitudes of the net assimilation rates, for these do not appreciably affect the value of the compensation point (Blackman and Wilson, 1950).

For the sunflower and buckwheat experiments (expts. 1–7 and 27–30) general curves showing the mean effects of light intensity on relative growth rate can be derived from the *mean* values of (i) the compensation point, (ii) the leaf-area ratio in daylight, (iii) the leaf-area ratio extinction point determined from the last sampling occasion, and (iv) the net assimilation rate in full daylight. Similar curves can be obtained from the comparable data for *T. subterraneum* and for *G. urbanum*, where it is assumed that at the end of the long experimental period (21 days) full modification of the leaf-area ratio at the different light levels had taken place.

The curves of relative growth rate against the logarithm of light intensity of these four species, together with the relevant data for net assimilation rates, compensation-points, leaf-area ratios, and extinction points, are given in Fig. 23. Considering first the relative growth-rate curves, it is seen that the species differ widely in the light level at which a maximum relative growth rate is attained. For *G. urbanum* the maximum is at 0·54 daylight, for *H. annuus* at 0·71, for *F. esculentum* 0·95, and for *T. subterraneum* 1·78. Since the compensation-point values only vary between 0·08 and 0·94 daylight, it follows that the progressive shift in the light-intensity value for maximum growth rate from *G. urbanum* to *T. subterraneum* is largely controlled by the change in the value of the extinction point. The extinction point in turn is dependent on a combination of the leaf-area ratio in full daylight and the slope of the linear regression linking leaf-area ratio with the logarithm of light intensity. Thus, the difference in the light value for maximum growth rate between *G. urbanum* and *F. esculentum* is dependent on a difference in the leaf-area ratio in full daylight (98 as against 183) and not on a difference in slope of the regression lines (*b*) for leaf-area ratio, since these lines are approximately parallel. In contrast, the difference between *G. urbanum* and *H. annuus* is linked with a change in slope (177 as against 128) rather than the leaf-area ratio in full daylight (98 and 100 cm./g.). It is also evident that the high light requirement of *T. subterraneum* is due to the small increases in leaf-area ratio which result from shading.

The magnitude of the relative growth rate at any light intensity is dependent on the net assimilation rate and the leaf-area ratio, and the data for *H. annuus* and *F. esculentum* given in Fig. 23 confirm the conclusions reached in expts. 27 and 30 that the higher leaf-area ratios of buckwheat more than offset any difference in the assimilation rates in determining the greater relative growth rate of *F. esculentum*.

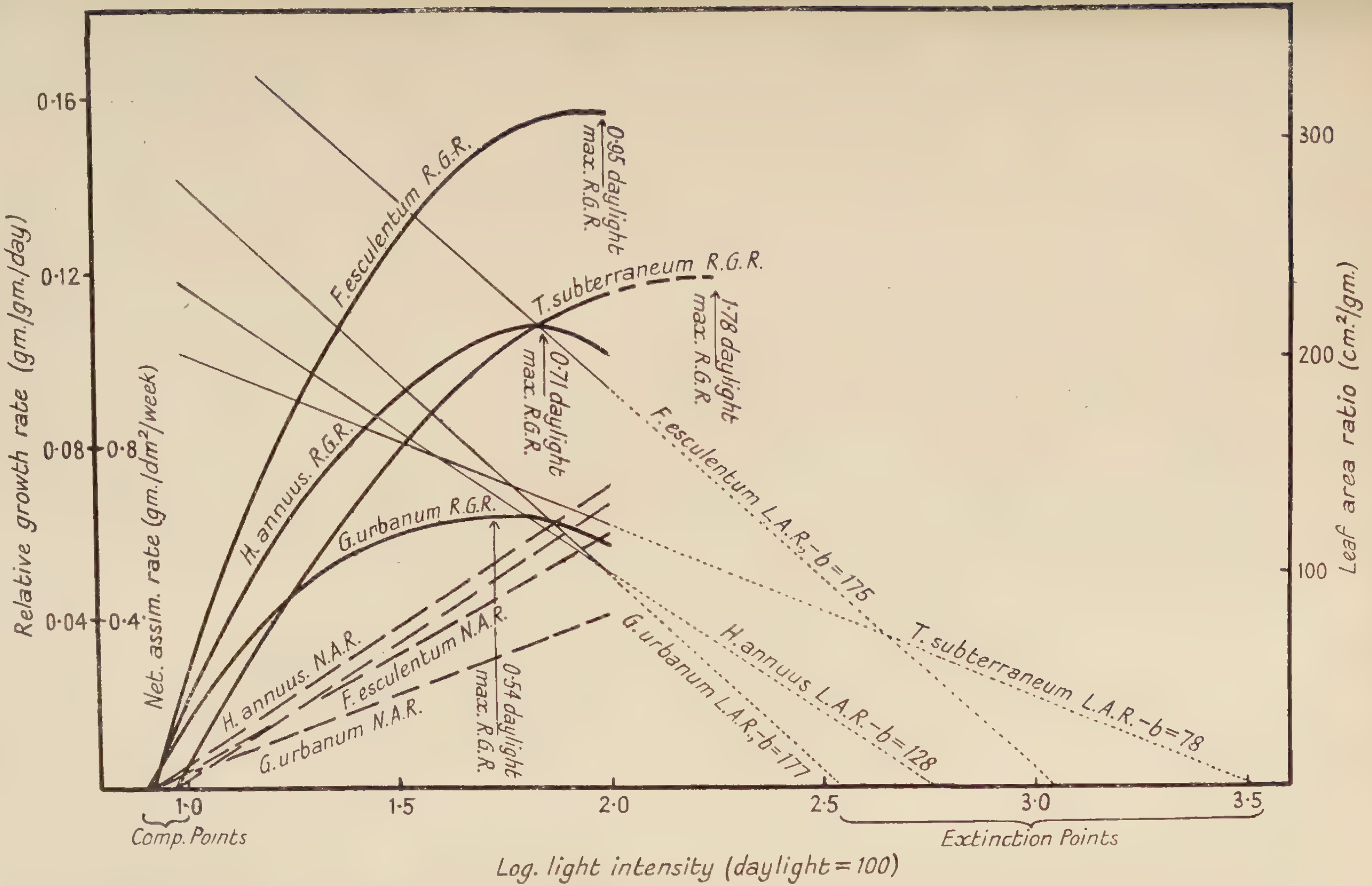

FIG. 23. *Fagopyrum esculentum*, *Trifolium subterraneum*, *Helianthus annuus*, *Geum urbanum*. The interrelationships for different species between net assimilation rate (N.A.R.), leaf-area ratio (L.A.R.), relative growth rate (R.G.R.), and the logarithm of light intensity. Arrows indicate the light intensity at which the relative growth rate will be maximal.

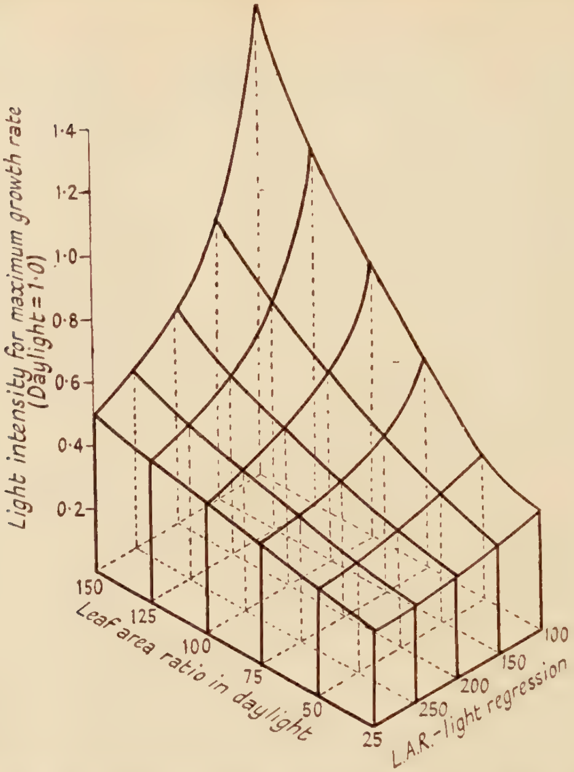

FIG. 24. The combined effects of varying leaf-area ratio in full daylight and varying slope of the linear regression linking leaf-area ratio with the logarithm of the light intensity on the level of light intensity at which the relative growth rate will be maximal for a species with a postulated compensation point of 0·08 daylight.

TABLE III

Differences between Species in the Factors governing the Light Intensity for Maximum Relative Growth Rate

	Light intensity for maximum growth rate (daylight = 1·0).	Leaf-area ratio in full daylight cm^2./g.	Slope of regression, leaf-area ratio/ log. light intensity	Compensation point (daylight = 1·0)	Extinction point, log. light scale (daylight = 2·0)	Rel. growth rate in daylight g./g./day
Hordeum vulgare	0·70	117	252	0·178	2·44	0·125
Solanum dulcamara	0·72	150	176	0·070	2·88	0·035
Lycopersicum esculentum	0·82	213	236	0·081	2·92	0·135
Vicia faba	0·90	75	91	0·114	2·85	0·042
Pisum sativum	1·29	116	88	0·085	3·29	0·062
Tropaeolum majus	1·70	143	82	0·081	3·55	0·053
Geum urbanum	0·54	98	177	0·080	2·55	0·058
Helianthus annuus	0·71	100	128	0·083	2·78	0·101
Fagopyrum esculentum	0·95	183	175	0·081	3·05	0·166
Trifolium subterraneum	1·78	120	78	0·094	3·53	0·115

Although the data are less accurate for the remaining species, the same type of analysis can be applied in assessing the factors which govern the level of light intensity at which the relative growth rate will be maximal, and the relevant information is given in Table III. The results indicate that *P. sativum* and *T. majus* are similar to *T. subterraneum* in their high light requirements, and it can again be concluded that this is due to a combination of a relatively high leaf-area ratio in full daylight with a low value for the regression of leaf-area ratio on the logarithm of the light intensity.

Although the optimal light values for *H. vulgare*, *S. dulcamara*, and *H. annuus* are very similar, the characteristics of the species in response to shading are different. Both *S. dulcamara* and *H. annuus* have similar values for the compensation point, and although *S. dulcamara* has a higher leaf-area ratio in full daylight, this is offset by the greater change in leaf-area ratio caused by shading; in consequence, the extinction-point values are approximately equal and therefore so are the values for the optimal light intensity. Between *H. annuus* and *H. vulgare* the higher compensation point of barley is largely counterbalanced by the greater increase in leaf-area ratio induced by shading and therefore a small value for the extinction point. Lastly, *V. faba* is somewhat exceptional since in this instance the optimal light value is determined by a low leaf-area ratio in daylight combined with little adaptation to shading.

In considering the results for *T. majus*, *S. dulcamara*, and *V. faba* it must be emphasized that the experiments were carried out in the autumn and that the relative growth rates are low (see Table III). In consequence, under these conditions full adaptation of the leaf-area ratios to the different light levels may not have taken place at the end of the experimental period, and this would lead to spuriously high values of the extinction point. The results for expt. 21 with *F. esculentum* indicate that in the autumn (September 25–October 13) the extinction point and the optimal light intensity may be higher than during periods of longer days and higher temperatures, for the values in this instance were 3·64 for the extinction point and 1·86 daylight for a maximal growth rate; figures very different from the values of expts. 20, 27, 29, and 30, where the variation in extinction point was from 2·91 to 3·21, and for the optimal light intensity the range was 0·78 to 1·10 daylight.

For *S. dulcamara* an intermediate sample was taken for the determination of the leaf-area ratios, and it is possible to examine the change with time in the value of the extinction point. For the first period it was 4·0, for the second period 3·26, and for the last sampling occasion 2·88. Although it is evident that much modification in the leaf-area ratio had taken place during the experiment, the differences between the last two figures are larger than those for the sunflower experiments (Table II), and it is therefore possible that under conditions of more rapid growth a somewhat lower value would be obtained, and that in consequence the light intensity for maximum growth would be less. The same overestimation of the extinction point and optimal light intensity may also have occurred for both *V. faba* and *T. majus*.

For all the ten species of this investigation and also for *Scilla non-scripta* (Blackman and Rutter, 1949), *G. urbanum* has the lowest intensity for optimal growth, namely, 0·54 daylight. From general ecological observation of the ground flora in some types of woodland this minimum figure seems surprisingly high, and it is worth while considering some of the characteristics which will enable species to grow best in deep shade. For any given values of the compensation point, the leaf-area ratio regression on light intensity, and the ratio in full daylight it is possible to calculate the light intensity at which the growth rate will be maximal, and such calculations have been made, assuming a constant compensation point of 0·08 daylight with a leaf-area ratio in full daylight varying from 25 to 150 cm.^{2}/g. and a regression of leaf-area ratio on the logarithm of the light intensity ranging from 100 to 300. These results are represented graphically in Fig. 24. It is seen that if a leaf-area ratio of *V. faba* (75 cm.^{2}/g.) in full daylight is combined with a leaf-area regression of *H. vulgare* (250: see Table III), then the optimal light intensity for growth is 0·4 daylight. To achieve optimal intensities appreciably below this level it is necessary to postulate a much lower leaf-area ratio in full daylight, e.g. a combination of 25 cm.^{2}/g. with a leaf-area ratio regression of 300 will result in maximum growth at 0·31 daylight. A leaf-area ratio in full daylight of this magnitude may be exceptional in most annuals, but in the perennial *S. non-scripta* ratios of this order have been found (Blackman and Rutter, 1950). If, however, such a low leaf-area ratio in full daylight is combined with a high leaf-area ratio regression, viz. 200–300, then the extinction point would be between 1·20 and 1·35 of full daylight, i.e. the leaf-area ratio would theoretically be reduced to zero at light intensities slightly above daylight. Under these conditions, some disturbance of leaf production in daylight might well be expected. In this connexion Goodall (private communication) has found that the seedlings of cocoa (*Theobroma cacao*), which demands shade for successful production in West Africa, fail to produce normal leaves when exposed to daylight.

Although for all the eleven species the mean compensation-point values have not fallen below 0·07 daylight, it is of some interest to calculate the optimal intensity for growth at lower compensation points; this has been done in Table IV for plants with the shade characteristics of a low leaf-area ratio in daylight, postulating compensation points of 0·05 and 0·02 of daylight.

Inspection of Table IV reveals that even with the lowest values for the compensation point and leaf-area ratio in daylight and the highest value for the leaf-area regression the minimum light level for an optimal growth rate is 0·15 daylight. Such a level is well above the light intensity of closed deciduous woodland in England once the canopy has fully expanded (Blackman and Rutter, 1946), and this will also hold for closed woodland in other parts of the world. On the available evidence, therefore, it must be assumed that when the canopy of closed woodland is fully expanded the growth of the ground flora is severely restricted by the low light intensity.

TABLE IV

The Interrelationship between Variations in (i) Compensation Point, (ii) Leaf-Area Ratio in Daylight, and (iii) the Slope of the Regression Line of Leaf-Area Ratio on the Logarithm of the Light Intensity, on the Level of Light Intensity for Maximal Relative Growth Rate

Compensation point (Daylight = 1·00)	Leaf-area ratio (daylight) (cm.^{2}/g.)	Light intensity for maximal relative growth rate (daylight = 1·0) Regression of leaf-area ratio on log. light (cm.^{2}/g./log. daylight—log. 0·1 daylight)					
		50	100	150	200	250	300
0·08	25	0·53	0·38	0·35	0·33	0·32	0·31
0·05	25	0·42	0·30	0·28	0·26	0·25	0·25
0·02	25	0·26	0·19	0·17	0·17	0·16	0·15
0·08	50	0·89	0·50	0·42	0·38	0·35	0·34
0·05	50	0·71	0·40	0·33	0·30	0·28	0·27
0·02	50	0·45	0·25	0·21	0·19	0·18	0·17
0·08	75	1·66	0·68	0·50	0·44	0·40	0·38
0·05	75	1·32	0·54	0·40	0·35	0·32	0·32
0·02	75	0·83	0·34	0·25	0·22	0·20	0·19

The data of Table IV emphasize what is also evident in Fig. 24, namely, that variations in the slope of the regression line for leaf-area ratio between 50 and 150 bring about by far the largest changes in the optimal light intensity.

Although the experiments concerned with the interrelationship between light intensity and nutrient level are only preliminary, they are a first approach to the manner in which nutrient supply may operate. From the results of experiments carried out on an adjacent area at Slough to expts. 11 and 12, it is likely that the control plants lacked nitrogen, phosphorus, and potassium, and it is therefore not possible to assess the individual effects of the nutrients on the net assimilation rate and leaf-area ratio. However, although the net assimilation rate is increased at the higher light intensities there is no change in the value of the compensation point, whereas where there is a nutrient effect on leaf-area ratio this is largely independent of the light intensity (see Fig. 10). It therefore follows that while the compensation point remains constant, the extinction point and therefore the value of the light intensity for optimal growth will be increased. Thus it is evident that a high nutrient level, which changes the leaf-area ratio, will be coupled with a greater light requirement for optimal growth to take place.

Lastly, there remains for discussion what bearing these results may have in the interpretation of the ways in which the light factor operates in environments with different limits of day length, mean light intensity, and temperature. Since both for *H. annuus* and *F. esculentum* the linear and curvilinear relationships with the logarithm of light intensity hold for experiments carried out between May and early September, it is clear that at least for these species the limits of light intensity, day length, and temperature cannot be critical. For example, with *H. annuus* the day length has ranged from 16·5 hours (expts. 1 and 5) to 12·25 hours (expt. 2), while the mean daily temperature limits varied from a combination of 27° C. maximum and 16·2° C. minimum for the second period of expt. 3 to 15·8° C. and 4·2° C. for the first period of expt. 6. Again, for *F. esculentum* the day length varied from 11·25 hours (expt. 21) to 16·75 hours (expt. 30), and whereas in expt. 29 the mean maximum and minimum day temperatures were 24·2° and 13·7° C., they were, in the first period of expt. 21, 18·0° and 7·1° C.

In the previous paper (Blackman and Wilson, 1950) the general conclusion was reached that during the *summer* months the net assimilation rate of *H. annuus* is largely independent of temperature, but logarithmically proportional to the light energy received, and the results of further experiments now in progress appear to confirm this conclusion. Shirley (1935) states that during midsummer the diurnal energy received per day in sub-arctic and temperate regions is little different from that in the tropics, while Heath and Gregory (1938) were the first to emphasize that on the then available evidence the net assimilation rate varies within relatively narrow limits for tropical and temperate climates. Subsequently it has been shown by Watson (1947) and in these investigations that there are specific differences in the net assimilation rate, but nevertheless the variation is not large. Thus it would seem that in widely different environments net assimilation rate will be logarithmically related to variations in the diurnal light energy.

For buckwheat and sunflower it can also be concluded that during the summer months the effects of shading on the leaf-area ratio cannot be highly correlated with temperature. It is also apparent that the linear relationship between leaf-area ratio and a logarithmic decrement in light intensity holds for a considerable variation in the day length. Such an independence could be explained on the same basis as that advanced for the changes in net assimilation rate, namely, that the effects of shading on the leaf-area ratio are due to a reduction in the total light energy received. On the other hand, an alternative hypothesis can be advanced that within a range of day length which allows of active growth the changes in leaf-area ratio brought about by shading result from a decrease in the light intensity rather than a diminution in light energy. In consequence, variations in day length would mainly operate by varying the level of the maximum intensity, i.e. the intensity received by the unshaded plants would vary with day length, the intensity being normally greatest when the days are longest.

To test directly this second assumption experiments have yet to be undertaken, but there is some indirect evidence that the leaf-area ratio is linked with light intensity. For example, in expts. 3, 21, and 30 (Figs. 4, 13, and 22) between the first and second experimental periods the differences in net assimilation rate at each light level are small, yet the slope of the leaf-area ratio regression in the second period is steeper than in the first. Again, in other investigations which have yet to be reported changes in leaf-area ratio have also been found to be to some extent independent of the value of net assimilation rate. Briefly, these experiments have been concerned with the effects of transferring sunflower seedlings grown for a period in the open or under one shade level to other levels of higher or lower intensity. It has been found that whereas for net assimilation rate in the second period there are no 'after effects' of the initial light levels, the pretreatments have large effects on the subsequent changes in leaf-area ratio. When the plants are transferred to a higher light level there is a progressive reduction with time in the leaf-area ratio, while in contrast there is an increase if the plants are more heavily shaded. Thus, in the second phase within each light treatment, plants may have the same net assimilation rate, but the leaf-area ratio may be relatively constant, rising or falling according to the light treatment in the initial phase.

Irrespective of whether total light energy or mean light intensity are operative in determining the leaf-area ratio, it is evident that the effects of light level on net assimilation rate and leaf-area ratio are compensatory in that high intensities raise the net assimilation rate but repress the leaf-area ratio. In consequence, for species with a maximum growth rate at a light intensity approaching full daylight, over a considerable range of light intensity and day length the relative growth rate is more or less constant. Indeed, it can be advanced that since the varieties of sunflower grown in this investigation and selected for seed production in England have also been productive in Europe, Morocco, Nigeria, Kenya, Tanganyika, South Africa, and southern Australia, the light factor will operate in the same compensatory way in all these countries.

The fact that a single variety of sunflower can effectively produce seed from the tropics to northern temperate zones is exceptional in that it indicates a wide tolerance of temperate conditions. Certainly *H. annuus* appears to be in a different category from *L. esculentum*, which Went (1944, 1945) has shown demands well-defined limits of day and night temperatures for growth and fruit production. However, for this species, Went has emphasized that the wide distribution is dependent on physiological differences between varieties. The data of Table II indicate that in the open the tomato plants were not growing under optimal conditions of temperature since only in the greenhouse did the relative growth rate (0·199 g./g./day) approach the maximum value of 0·24 found by Goodall (1945) in his studies of the growth under glass of young tomatoes at different periods of the year. On the other hand, in spite of this temperature limitation, the linear relationships between net assimilation rate and leaf-area ratio and the logarithm of the light intensity still hold (see Fig. 16).

If the diurnal light energy at midsummer is relatively constant between the tropics and the sub-arctic, and if a favourable but not necessarily an optimal environment is a prerequisite for the compensatory effects of light intensity to operate on leaf-area ratio and net assimilation rate, then it is of some interest to compare the growth rates in these experiments with those found for other species and other environments. Goodall (1945) has summarized the results of earlier workers and quotes a maximum relative growth rate of 0·176 for *H. annuus* in Germany as against a range of 0·063 to 0·174 in this investigation. For *H. vulgare* the rate of 0·125 (expt. 29) is similar to the maximum of 0·117 given by Gregory (1926). In the *F. esculentum* experiments the growth rate varied from 0·094 to 0·187 and the maximum value is amongst the highest figures yet recorded. In Australia Ballard and Petrie (1936) have observed a growth rate of 0·19 for *Andropogon sudanensis*, Petrie et al. (1939) give a figure of 0·155 for *Nicotiana tabacum*, and Williams (1946) 0·076 for *Phalaris tubersa* grown in a greenhouse. For German investigations Goodall (1945) quotes 0·147 for *Cannabis gigantea*, 0·15 for *Zea mays*, 0·181 for *Sinapis alba*, 0·195 for *Helianthus macrophyllis giganteus*, and 0·205 for tobacco. In Great Britain the maximum growth rates for mangolds and sugar-beet—0·104 to 0·115 (Watson and Baptiste, 1937)—are less than the rate for *T. subterraneum* (0·129), while in view of the unfavourable conditions in expt. 30 it is not unexpected that the growth rate of 0·06 for *P. sativum* is less than the maximum rate 0·11 determined by Brenchley (1920).

For warm climates there is a great paucity of information and most data are available for the shoot growth of cotton. Inamdar et al. (1925) observed in India a maximum of 0·129, Heath (1937) gives 0·101 for South African conditions, while Crowther (1934) records in the Sudan a lower maximum of 0·061 for irrigated plants. Under American conditions, according to Hammond and Kirkham (1949) the maximum growth rate for soya-beans was 0·088 and for maize (shoots only) 0·159.

From these results, admittedly inadequate, because workers cannot have published much relevant data, the tentative conclusion is reached that plants do not grow faster in the tropics than in northern temperate zones. In fact, at present the highest maximum growth rates have all been recorded for temperate regions, but since there are many more observations for the cooler climates this difference may be an artifact. On the other hand, with the twin assumptions that net assimilation rate is logarithmically related to total light energy and leaf-area ratio is correlated with both light intensity and total light energy, then a greater relative growth rate might be expected in the longer days of the temperate zones with their equal energy but lower mean light intensities. In this connexion, the highest growth rate of all has been recorded by Goodall (1945) for the tomato, a plant of tropical origin grown in a more northerly latitude but protected from the lower temperatures by cultivation in a greenhouse. It would be exceedingly interesting to know what would be the growth rate of the tomato under glass in the sub-arctic at midsummer. Likewise it would be of great interest to establish for sub-arctic environments what are the net assimilation rates and growth rates of plants with relatively low temperature requirements, such as peas and barley, which are capable of maturing seed in the short summers of such areas as northern Sweden. It may well be that the relative growth rates will be still higher than those ruling in the temperate zone or in the tropics.

SUMMARY

Since relative growth rate is the product of net assimilation rate and leaf-area ratio (leaf area/plant weight), it follows that if the effects of shading on

net assimilation rate and leaf-area ratio can be expressed mathematically, then the relationship between light intensity and relative growth rate can be derived from the product of the two mathematical expressions.

In an analysis of the effects of shading on vegetative growth, some twenty pot experiments and eleven field tests have been carried out in which by means of screens (butter-muslin, perforated metal, or wire gauze) the plants have been subjected to light intensities ranging from full daylight to 0·1 daylight. The chief species investigated were *Helianthus annuus* and *Fagopyrum esculentum*, but experiments were also undertaken with *Trifolium subterraneum*, *Hordeum vulgare*, *Vicia faba*, *Pisum sativum*, *Lycopericum esculentum*, *Tropaeolum majus*, *Geum urbanum*, and *Solanum dulcamara*.

For all species it has been established that both the changes in net assimilation rate and leaf-area ratio are linearly related to the logarithm of the light intensity. In consequence, the relationship between the logarithm of light intensity (x) and relative growth rate (y) should be curvilinear, i.e. $y = ax^2 + bx + c$, and there is a close agreement between the observed and calculated values. It is therefore possible to determine the light intensity at which the growth rate is maximal with a new precision.

In two of the eleven field trials with *H. annuus*, the combined addition of nitrogen, phosphorus, and potassium had significant positive effects. For net assimilation rate and relative growth rate, but not for leaf-area ratio, there were significant interactions between nutrient level and light intensity, the nutrient effects being greatest at the higher light intensities.

For species of shady habitats (*G. urbanum*, *S. dulcamara*) neither the levels of net assimilation rate nor the compensation-point values—obtained by extrapolation of the linear regressions—are very different from those of the eight species from open situations, e.g. *H. vulgare*, *P. sativum*, *F. esculentum*. Nevertheless, the optimal intensity at which the relative growth rate is maximal varies widely between species: it is 0·5 daylight for *G. urbanum*, 0·7 for *H. annuus*, full daylight for *F. esculentum*, while for *T. subterraneum* the calculated value is 1·8 daylight.

These specific differences in the effects of shading on the growth rate are more dependent on the variations in leaf-area ratio than on the changes or magnitude of the net assimilation rate. Changes in nutrient level may operate in shifting the optimal light intensity by altering either the compensation point or the leaf-area ratio, but in the present experiments only variations in the ratio have been observed.

On the basis of this analysis of the light factor, it is possible to assess theoretically the characteristics of 'sun' and 'shade' plants. A shade plant can best be redefined as a species for which a reduction in the light intensity causes a rapid rise in the leaf-area ratio from an initial *low* value in full daylight. Conversely, a sun plant combines a high leaf-area ratio in full daylight with little change in the ratio at lower light intensities.

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The Composition of the Gas contained in the Vesicles of *Fucus vesiculosus*

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With three Figures in the Text

SUMMARY

Analysis of the gases contained in the vesicles of *Fucus vesiculosus* showed that their oxygen content may be markedly enhanced by photosynthesis.

Under conditions of exposure to the air it was found that the oxygen content rarely fell much below 20 per cent. even after prolonged periods in the dark.

The carbon dioxide content of the vesicular gas varied from 0 to 2·5 per cent. and appeared to bear no relationship to metabolic activity.

The results as a whole suggested that vesicle formation may possibly be conditioned by the intensity of photosynthetic activity.

INTRODUCTION

THE investigation described in the present paper was designed to amplify certain observations made by Burrows (1950). In the course of work upon the Manx fucoids she has shown that the formation of vesicles on the fronds of *Fucus vesiculosus* is directly related to the roughness of the water washing the plants during the tidal period. Thus, when growing on steep rock surfaces on exposed coasts the plants are almost entirely evesiculate, whereas vesicles are profusely developed on plants growing in quiet bays and inlets. These findings of Burrows are in agreement with those of Cotton (1912) for Clare Island, of Richard (1925), and with the observations of Knight and Parke (1950). On the basis of his own observations Richard has suggested that the vesicles are to be regarded simply as floatation bladders. He considers that the vesicles contain normal air and that their contents are not affected by respiration. In view of the results of other workers on the composition of the internal gases of various brown seaweeds (Lucas, 1912; Zeller and Neikerk, 1915; Colla, 1931, 1935; Damant, 1936) this explanation seemed to the authors to be unlikely. Furthermore, the work of Stocker and Holdeheide (1938) has shown that *Fucus vesiculosus* behaves as a sun plant. Hence it seemed probable that the presence or absence of vesicles might be conditioned by the extent of photosynthesis during the period of active growth in which the vesicles are laid down. Thus if gaseous distension of the tissues due

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to active photosynthesis plays any significant role during the differentiation of these vesicles, then it might be expected that they would occur more abundantly in clear sheltered water than in rough water in which, because of turbidity due to suspended air-bubbles, the rate of photosynthesis would be comparatively lower by reason of diminished light intensity.

Determinations were therefore made of the oxygen content of the vesicular gases of *Fucus vesiculosus* in order to ascertain the extent to which this is affected by external conditions of light and exposure to the atmosphere. Carbon dioxide content was determined at the same time; and lastly, in the light of the work of Langdon and Gailey (1920) on *Nereocystis*, tests for the presence of carbon monoxide were also made on the vesicular gases.

EXPERIMENTAL METHODS

In experiments designed to assess the effects of respiration and photosynthesis on the composition of the vesicular gases the following procedure was adopted. The experimental plants were subjected to two pairs of alternative treatments prior to the extraction and analysis of their vesicular gas. In the four combinations of these treatments, illuminated or darkened plants were either exposed to the atmosphere or just submerged beneath the water surface in a tank of sea-water. Where dark conditions were required, small tanks covered with sacking were used; and when the plants were both darkened and submerged, sea-water was run through the tank continuously during the experimental period. The illuminated plants were either suspended from the wall of a large open-air tank with the tips of the fronds just above the water surface, or alternatively they were placed in a large wire tray fastened to the wall and immersed just beneath the surface.

Each pair of treatments requiring either light or darkness was carried out simultaneously. The plants used in any particular experiment were gathered at the same time from the beach at Port Erin, I.o.M. Two samples of four plants each were reserved for immediate gas extraction from the bladders, and the remaining plants were then subjected to the appropriate treatment over a period in which sampling was usually carried out at 3-hourly intervals. Each sample consisted of four randomly selected plants, and was once replicated for each treatment at each successive time interval.

Gas extraction was performed in a simple manner. The vesicles were pricked under sea-water and the gas was allowed to escape into a small inverted test-tube filled with sea-water. Clearly this method was not ideal, but errors due to the solubility of the vesicular gases in sea-water were minimized by using the smallest practicable volume of sea-water, and transferring the gas at once to a sampling tube in which it could be stored over mercury. At any one sampling time, the gas from all the vesicles of four plants was collected, and a small amount of this transferred to a sampling tube.

Analysis of the gas samples for oxygen and carbon dioxide was carried out by a modification of the Bonnier and Mangin apparatus (Vallance & Coult, 1951). Initial tests on gas samples extracted from plants which had been kept enclosed and in darkness for a period of 2 days showed that carbon monoxide was present. The method used, that of Heilmeyer (1943), involved the spectroscopic examination of samples of blood, after shaking them with the gas samples in question. Subsequent similar qualitative tests together with quantitative tests in the gas analyser, using cuprous oxide and β-naphthol dissolved in sulphuric acid as the absorbent, gave negative results. In view of these results no further work on the carbon monoxide content of the vesicles was carried out and after the removal of carbon dioxide and oxygen it was assumed that the residual gas was nitrogen.

EXPERIMENTAL RESULTS

Table I shows the composition of the vesicular gas of a series of groups of plants of *Fucus vesiculosus* immediately after gathering. The plants were gathered at different times of the day over a period of 6 days during April 1950; they had been left uncovered by the tide for varying periods of time. The figures given are in general agreement with those of workers on other seaweeds in that oxygen concentrations of up to 33 per cent. were obtained and in that little or no carbon dioxide was present.

TABLE I

Collected Beach Records. Gaseous Composition of Vesicles in Plants sampled just after Gathering

Date of collection.	Time of gathering (B.S.T.).	Percentage oxygen.	Percentage carbon dioxide.	Comments.
16/5/50	14.30	25·6	0·0	Overcast, cloudy
	15.30	23·2	0·2	,, ,,
	16.30	22·2	0·2	,, ,,
		26·4	0·3	
	18.30	25·9	0·0	,, ,,
		24·3	0·2	
17/5/50	18.00	31·1	1·9	Overcast, light cloud
		30·2	1·1	
		29·5	2·0	
		25·1	2·2	
19/5/50	09.30	26·3	0·0	Light cloud
		22·0	0·9	
	17.00	30·5	0·7	,, ,,
		33·9	0·8	
20/5/50	10.00†	28·6	0·3	,, ,,
		15·2	0·3	

† Sampled 1½ hours after collecting and storage in dark.

These observations cannot in themselves adequately illustrate the effect of the change over from light to darkness and vice versa on the composition of the vesicular gases. In addition they do not show the effects of submergence and exposure to the air. The next three figures illustrate the effects of these factors on the composition of the gas. Fig. 1 shows the effect of an initial dark period and subsequent illumination on the composition of the vesicular gases. It will be seen that at the time of gathering the mean oxygen concentration was 27·5 per cent. After 14 and 14½ hours respectively in the darkness the oxygen content diminished to 21 per cent. in the exposed plants and to 19·4 per cent. in the submerged plants. During the illumination of the remainder of the experimental plants on the following day the oxygen concentration rose in the submerged plants to nearly 29 per cent., while in the exposed plants, even though these became somewhat desiccated, the oxygen concentration increased to 24·5 per cent. after 6 hours' illumination. The fall in percentage oxygen after the 6th hour of illumination which followed was almost certainly due to the clouding over of the sun. The results given in Fig. 1 clearly show the effect of photosynthesis on the vesicular oxygen content, but do not show whether or not this may be markedly depleted during prolonged respiration in the dark.

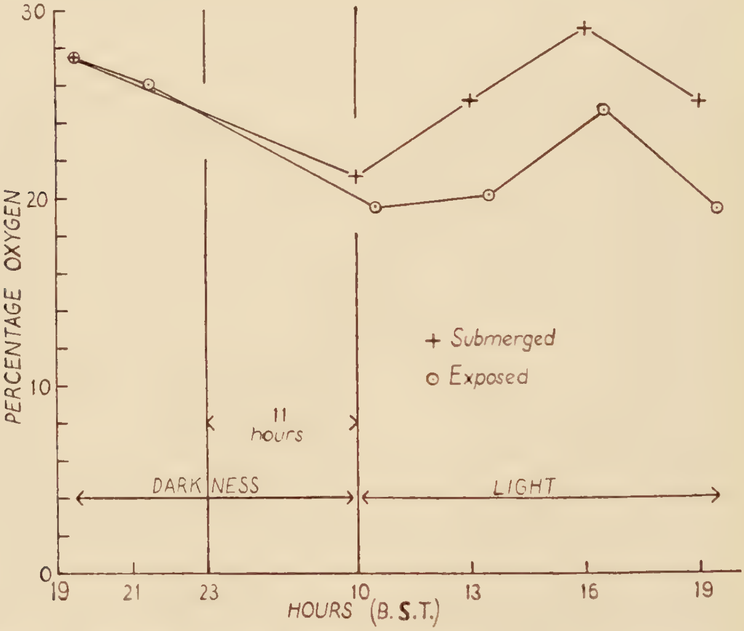

Fig. 1. The changes in the oxygen concentration of the vesicular gas of *Fucus vesiculosus* during a period of darkness followed by illumination.

Fig. 2 shows the data of a further experiment on the effect of darkness on the vesicular gas. In this case plants were collected from the beach and maintained in darkness whilst being either submerged or exposed to the air. During the first 8½ hours the fall in oxygen concentration was much as expected, the mean value diminishing from 28 per cent. to 21 per cent. and 18 per cent. in the exposed and submerged series respectively. After this time the remaining plants of both series were placed in large closed museum jars in darkness. In the first series, where very little sea-water was present in the closed jar, the oxygen content did not change greatly from the value given even when the plants were left until 15.00 hours on the next day. In the second (submerged) series, however, where the plants in the closed jar were covered with sea-water, the oxygen content during the same period dropped to about 6 per cent. It was remarkable that even in this last series the carbon dioxide concentration was less than 2 per cent.

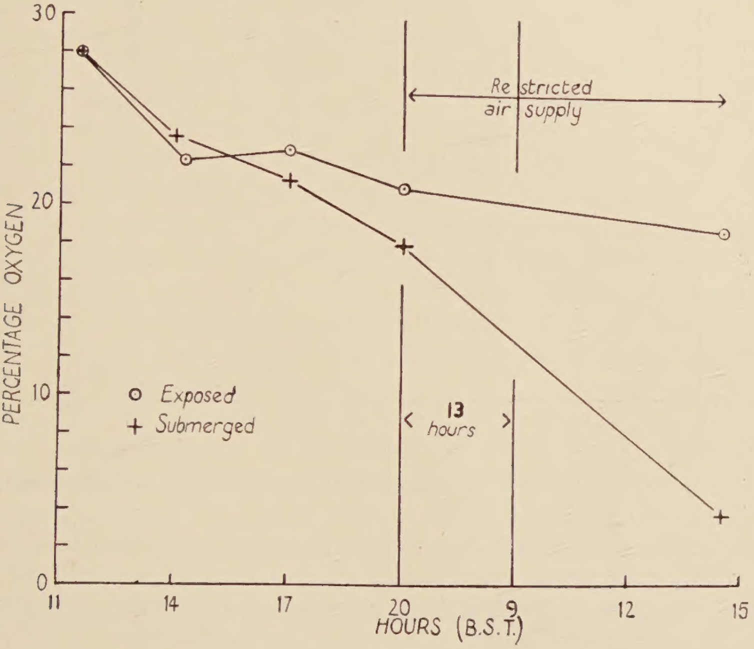

FIG. 2. The effect of a prolonged period of darkness on vesicular oxygen content.

The last figure shows the changes in the amount of oxygen in the vesicles during an experiment in which the plants were maintained in daylight after gathering. This experiment showed once more that oxygen concentration increases most readily in illuminated plants that are just submerged. Comparable plants maintained in darkness until 11.00 hours on the following day showed a fall in oxygen content to 18·6 per cent. in the exposed series and 16 per cent. in the submerged series. In this last experiment, as in previous ones, the carbon dioxide concentration varied between 0 per cent. and 1 per cent., and could not be related to oxygen concentration.

In the previously mentioned experiments all the vesicles on a plant were sampled and so it was not possible to detect any differences which might have existed between the composition of the gases contained by the old and young vesicles. It seemed probable that some such difference might exist and so determinations were made to clarify this point. Plants were gathered from the beach at 09·00 hours and placed in the tank. They were allowed to remain there until 17.00 hours to allow for adequate photosynthesis and to reduce variation between individual fronds; the gases contained in the old and young vesicles were then extracted. Table II shows the results of the analysis of these gases. It will be seen from this table that in all replicates the oxygen concentration was higher in the old than in the young vesicles.

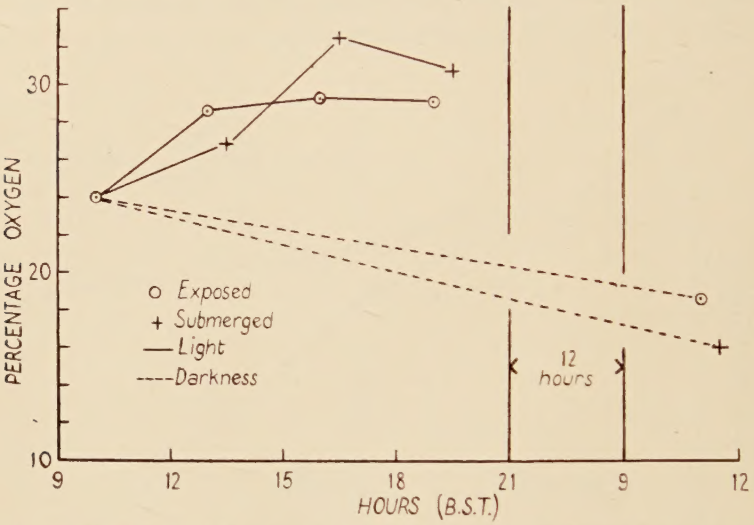

Fig. 3. The effect of illumination and darkness on vesicular oxygen content.

A final series of observations was carried out to determine whether there was any difference in the oxygen concentration of the gas contained in the young vesicles and in the so-called 'boursouflures' of Richard (1925). No difference could be established.

Table II

Composition of the Gas in Vesicles of Old and Young Fronds after Eleven Hours of Daylight

Old		Young	
Percentage oxygen.	Percentage carbon dioxide.	Percentage oxygen.	Percentage carbon dioxide.
28·7	0·2	27·3	0·4
28·0	0·2	22·9	0·3
29·0	0·2	26·5	0·5
28·9	0·2	25·3	0·6
Mean: 28·6	0·2	25·5	0·4

Discussion

It seems clear that the vesicles of *Fucus vesiculosus* are not merely floatation bladders as postulated by Richard. Their oxygen content would seem to be regulated by both photosynthesis and diffusion, so that in general it is maintained at above 20 per cent. in the light and in the neighbourhood of 20 per cent. in the dark, under conditions of adequate aeration. The difference in the oxygen concentration of the old and young vesicles may also be explained on a diffusion basis, for the rate at which oxygen diffuses across the thinner walls of the newly formed vesicles will presumably be greater than the rate across the thicker and more rigid walls of the vesicles of older parts of the plant. The range of variation in samples taken directly from the shore at the same time would seem to be primarily the result of differential shading, but in those cases where exposed plants are lying at the bottom of a pile the oxygen supply also may be restricted. This latter suggestion may account for the single field record in which a sub-atmospheric level for oxygen was obtained.

Other authors have recorded low values for the carbon dioxide content of the internal gases of the brown seaweeds similar to those recorded in the present paper for *Fucus vesiculosus*. In the case of *Fucus vesiculosus* there was no apparent correlation between carbon dioxide concentration and change in oxygen concentration. Furthermore, the low levels of carbon dioxide recorded might possibly have been due to some effective fixation mechanism, but it seems more likely, as has been suggested for oxygen, that diffusion tends to establish an equilibrium between the carbon dioxide content of the vesicles and that of the external environment.

The results of this investigation seem to lend weight to the suggestion that vesicle formation in quieter waters may be linked with a relatively high rate of photosynthesis. Oxygen production in the vesicles achieves its highest level in plants which have been in full daylight for the best part of a day, and no one who has handled these fronds through the day can fail to have been struck by the increasing tenseness of the vesicles as the gas pressure inside them mounts. There can be little doubt that *Fucus vesiculosus* runs closely parallel in this and other respects to *Ascophyllum nodosum* (see Damant, 1936). The organization of the vesicles of the former suggests that there may be a more or less controlled growth response to this feature of gas production and accumulation. The production of what Richard has called 'boursouflures' seems to the authors to be merely a less-well-defined opening-out of air cavities in the thallus consequent upon the production and accumulation of gaseous oxygen in the tissues as a result of photosynthetic activity sustained at a high rate. The decrease in intensity of the light incident at the surface of the plants growing in rough turbid waters may well account for both their somewhat stunted stature as well as for their normal failure to produce vesicles.

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